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Environmental pollution and nutritional quality modulate immune response of the wood mouse (*Apodemus sylvaticus*) through hormonal disturbances

Quentin Devalloir^{1,*}, Clémentine Fritsch¹, Yara Alchammas¹, Francis Raoul¹, Vincent Driget¹, Caroline Amiot¹, Shinji Ozaki^{1,2}, Nico van den Brink³, Renaud Scheifler¹

¹UMR 6249 Chrono-environnement, CNRS / Université Bourgogne Franche-Comté, Université de Franche-Comté 16 route de Gray, 25030 Besançon Cedex, France

²UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK

³Division of Toxicology, Wageningen University, 6700 EA Wageningen, The Netherlands

*Corresponding author: quentin.devalloir@univ-fcomte.fr

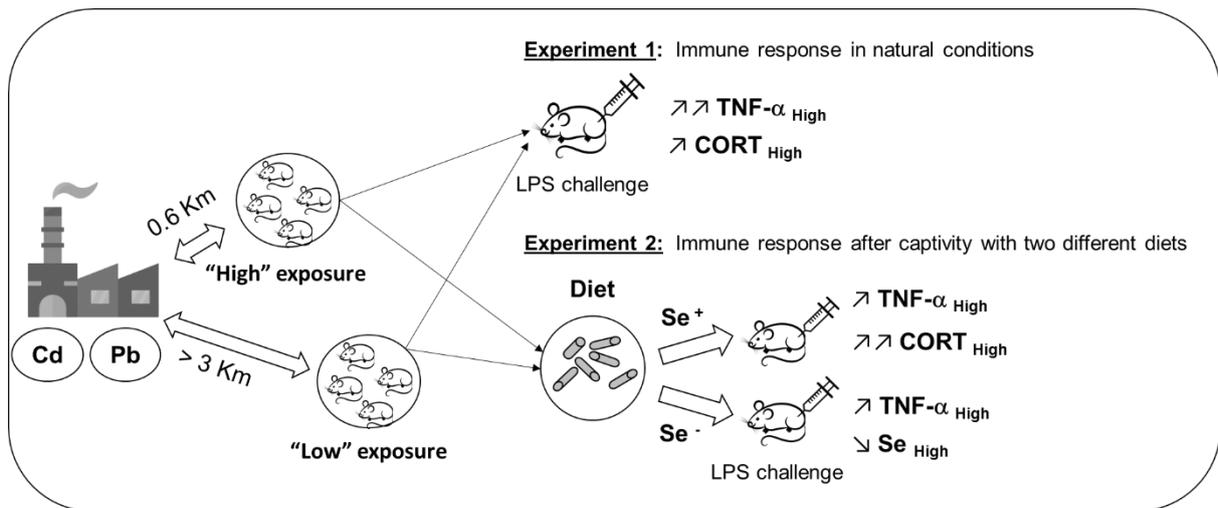
Abstract

Cadmium (Cd) and lead (Pb) are known to enhance immune cell damages and to decrease cellular immunity, promoting higher susceptibility to infectious diseases. Selenium (Se) is an essential element involved in immunity and reactive oxygen species scavenging. This study aimed at evaluating how Cd and Pb and low nutritional (Se) quality modulate immune response to a bacterial lipopolysaccharide (LPS) challenge in wood mice (*Apodemus sylvaticus*). Mice were trapped near a former smelter in northern France in sites of High or Low contamination. Individuals were challenged immediately after capture or after five days of captivity, fed a standard or a Se-deficient diet. Immune response was measured with leukocyte count and plasma concentration of TNF- α , a pro-inflammatory cytokine. Faecal and plasma corticosterone (CORT), a stress-hormone involved in anti-inflammatory processes, was measured to assess potential endocrine mechanisms. Higher hepatic Se and lower faecal CORT were measured in free-ranging wood mice from High site. LPS-challenged individuals from High site showed steeper decrease of circulating leukocytes of all types, higher TNF- α concentrations, and a significant increase of CORT, compared to individuals from Low site. Challenged captive animals fed standard food exhibited similar patterns (decrease of leukocytes, increase of CORT, and detectable levels of TNF- α), with individuals from lowly contaminated site having higher immune responses than their counterparts from highly polluted site. Animals fed Se-deficient food exhibited lymphocytes decrease, no CORT variation, and average levels of TNF- α . These results suggest (i) a higher inflammatory response to immune challenge in free-ranging animals highly exposed to Cd and Pb, (ii) a faster recovery of inflammatory response in animals lowly exposed to pollution when fed standard food than more exposed individuals, and (iii) a functional role of Se in the inflammatory response. The role of Se and mechanisms underlying the relationship between glucocorticoid and cytokine remain to be elucidated.

Keywords

Immunomodulation, trace metals, selenium, glucocorticoids, mammals

Graphical abstract



Highlights

- Cadmium, lead and selenium modulate immune response of wild and captive wood mice
- Animals from highly polluted site exhibited high inflammatory response
- Captive animals from lowly polluted site fed standard food showed high response
- Captive animals fed Se-deficient food exhibited low response
- Inflammatory responses were related to Se and might be mediated by corticosterone

Introduction

Severe changes have occurred in many habitats of free ranging mammal populations (e.g. fragmentation, habitat loss, pollution, reduction of food availability and/or quality...) turning their living area into a stressful environment. Wild rodents are also constantly exposed to infectious diseases (Strandin et al., 2018; Viney and Riley, 2017), making the immune system integrity and efficiency crucial to their health and fitness. Pollution and poor nutritional quality have been shown to have severe implication on both immune and endocrine systems (Acevedo-Whitehouse and Duffus, 2009). The role of the immune system is to eliminate pathogens while the endocrine system ensures peripheral organ functioning to maintain homeostasis (Wensveen et al., 2019). However, both maintain an equilibrium between pro- (immune) and anti- (endocrine) inflammatory responses to a stress, in order to avoid autoimmune damages or an increasing susceptibility to infections (Wensveen et al., 2019; Martin, 2009). In the purpose of the conservation of free ranging mammals, it is primordial to characterise the impact of both pollution and nutritional quality on the ability of organisms to respond to an infection, also known as immunocompetence. Wildlife diseases can be affected by pollutants by increasing pathogen prevalence and by decreasing immune function of the host (Acevedo-Whitehouse and Duffus, 2009).

Ensuring an efficient immune response against pathogens is central to fitness as it may reduce energetic cost allocated to reproduction for instance (Lochmiller and Deerenberg, 2000). The part of the energy allocated to immunocompetence belongs to the metabolic demand found in diet by organisms, which requires a sufficient amount of macro- and micro-nutrients (Downs and Stewart, 2014). Macronutrients (water, fibres, carbohydrates, lipids and proteins) maintain metabolic processes in providing vital elements (carbon, hydrogen, nitrogen...) and releasing energy. Micronutrients are idiosyncratic components of the immune system whose role varies from homeostatic regulators to constitutive elements of immunity (Allen and Ullrey, 2004; Gombart et al., 2020). Among them, dietary selenium (Se) was found to enhance immune cells functioning, proliferation and activity (Huang et al., 2012). Selenium is also a major antioxidant involved in reactive oxygen species (ROS) scavenging via selenoproteins and Se-dependent processes (Schweizer et al., 2016). In a previous study, we found that wood mice (*Apodemus sylvaticus*) free-ranging populations exposed to elevated levels of soil pollution by cadmium (Cd) and lead (Pb) had higher hepatic Se concentrations than control populations. We also found positive relationships between Se concentrations and haematological parameters (Powolny et al., 2022), suggesting that Se might play a protective role against Cd and/or Pb toxicity. In laboratory controlled conditions, Se has been shown to counteract immunosuppression as well as hepatic and renal oxidative damage induced by cadmium in orally exposed rats (El-Boshy et al., 2015).

Toxic metals like Pb and Cd are known to be particularly immunotoxic to mammals (Gera et al., 2015). Complete blood cell counts have commonly been used to assess health condition of individuals exposed to various pollution types including toxic metals (Luster et al., 1988). Leukocytes and derived white blood cells (*i.e.* monocytes, neutrophils, granulocytes) can help to interpret infectious status or to give a disease diagnosis, and have also been studied in a metal pollution context in the field (Rogival et al., 2006) or under controlled-condition exposure in the laboratory. Laboratory studies on mice and rats exposed to Cd or Pb showed that these metals dampened immune cell functioning, *e.g.* a decrease of lymphocyte proliferation or an increase of inflammatory markers (Gera et al., 2015). Both Cd and Pb can directly impair immune cell functioning by decreasing cell (*e.g.* T cells, NK cells) proliferation and activity (Gera et al., 2015; Wang et al., 2021). Cadmium and Pb exposure can enhance ROS

production which then, can indirectly affect nuclear factor- κ B pathway in promoting an increase of (pro-)inflammatory cytokines (*i.e.* tumour necrosis factor alpha (TNF- α), interleukin 1 Beta (IL-1 β), interleukin 6 (IL-6))(Hosseini-Khannazer et al., 2020; Liu et al., 2012). Toxic metals can enhance cell reactivity to self (autoimmunity) and modulate immune response against pathogenic agents (Kishikawa et al., 1997).

Both Cd and Pb are also known to disrupt the production/secretion of corticosterone (CORT) from the adrenal gland, CORT being the main glucocorticoid (GC) in rodents (Spackman and Riley, 1978). . As steroid hormones, GC production (in standard conditions) enables to decrease immune response in down-regulating immune system activity once infections are controlled. The production of GCs can reduce circulating lymphocytes, neutrophils, granulocytes and monocytes. Martin (2009) proposed a temporal framework of immune response to a stressor, mediated by stress hormones. In this framework, a first neuropeptide-dominated phase tends to accelerate the processes involved in the inflammatory response (within minutes to hours), and is followed by a second phase in which GC production down regulates the inflammatory response (within hours to months). The two phases constitute key regulators of the immune system. Thus, a close interaction exists between hormonal and immune response against stressors to prevent immune self-damages. The production of mice CORT by the adrenal gland can be enhanced by the release of pro-inflammatory cytokines (IL-6, TNF- α and IL-1) from immune cells (Silverman and Sternberg, 2012). Indeed, immune challenge of laboratory mice showed a combined increase of both TNF- α and CORT (Grion et al., 2007). Metabolic CORT disruption could have severe consequences on the regulation of the immune system, as low levels would maintain an up-regulation of the immune response after an episodic infection. A significant decrease of serum CORT was found in male Wistar rats exposed to chronic injection of Cd chloride, which could thus act as a neuroendocrine disruptor (Nishiyama and Nakamura, 1984).

This study aims at determining how toxic metals can modulate the immune response of wood mouse populations inhabiting polluted (mainly by Cd and Pb) areas near the former smelter of Metaleurop Nord (northern France). To test immunocompetence, individuals from highly and lowly polluted sites were subjected to an immune challenge enhanced by bacterial lipopolysaccharides (LPS). This experiment is hereafter referred to as Experiment 1. To test the potential role of nutritional quality, a sub-sample of the animals captured in the contrasted sites were maintained in captivity for 5 days, and fed with a standard or Se-deficient rodent food. This experiment is hereafter referred to as Experiment 2. We hypothesized that the LPS challenge in wood mice from highly polluted sites would promote higher levels of TNF- α due to neuroendocrine disturbances (as assessed by serum and faecal levels of CORT) mediated by Cd and Pb exposure. We also hypothesized that Se-deficient diet would shape discrepancies in wood mice inflammatory response to the challenge among contrasted levels of pollution.

Methods

Study site

The study was conducted in the surroundings of the former smelter Metaleurop Nord (northern France), which generated high levels of environmental pollution, mainly by Cd and Pb (Fritsch et al., 2010b, 2010a). Two groups of two (replicate) sites (of 25 ha), contrasted by their level of soil trace metal concentrations were studied, hereafter referred to as High and Low (Table S1). Median soil Cd and Pb concentrations in soils sampled in 2006 are of 1.4 and 116 $\mu\text{g/g}$ dry mass for Low site, and 59.3 and 1357 for High site, respectively (Douay et al., 2009; Fritsch et al., 2010a). An additional soil sampling performed in 2019 confirmed that total Cd and Pb concentrations did not change significantly between 2006 and 2019 and provided data for other elements like Se (Table S1, Scheifler et al., 2022).

Animal sampling and selection

From the 28th of September to the 13th of October 2020, 70 traplines of ten nonlethal INRA traps with dormitory boxes each spaced three meters apart were set for three to five consecutive nights. Dormitory boxes were filled with apple, hay, sunflower seeds, cat food and peanut butter to ensure survival of the animals for 24 h. Traps were checked every morning. The wood mouse (*Apodemus sylvaticus*) was retained as the animal model for this study considering its relevance for bioaccumulation of metals from soil and/or trophic transfer, its ubiquity and its ability to cope with captivity. Among the individuals trapped, individuals from other species than *A. sylvaticus* were immediately released, after checking their health status (no injury, not wet, apparent normal behaviour). Pregnant females and females exhibiting

suckling, as well as young individuals (i.e. below 15g, (Gelling et al., 2009)) of *A. sylvaticus* species were also released. Adult males and non-pregnant females were kept for the experiment. A total of 91 wood mice were trapped on which 46 of the 60 males and 13 of the 31 females caught were used for the experiment (Table 1).

Table 1 Sampling design, acronyms and number (in parenthesis) of wood mice caught on high and low levels of soil pollution. In bold are the acronyms that are used throughout text, tables and figures to refer to the different experimental groups.

Soil pollution levels	Study design	Type of food	Day 0 (D0)	Day 5 (D5)	Name of the tested groups	Experiment
High (29)	No captivity (6)	No food (6)	Challenged high (6)		H-LPS (6)	1
	Captivity (23)	Standard food (12)	Control high (H) (23)	Challenged high standard (12)	H-LPS-Se+ (12)	2
		Se-deficient food (11)		Challenged high Se-deficient (11)	H-LPS-Se (11)	2
Low (23)	No captivity (13)	No food (13)	Challenged low (13)		L-LPS (13)	1
	Captivity (13)	Standard food (7)	Control low (L) (13)	Challenged low standard (7)	L-LPS-Se+ (7)	2
		Se-deficient food (6)		Challenged low Se-deficient (6)	L-LPS-Se- (6)	2

Experimental design

The experiment design, sample size and names given to the various groups are summarized in Table 1 and Figure S1.

In experiment 1, a group of 19 individuals (6 from High site, and 13 from Low site) was immediately treated on the day they were captured. Individuals were immune-challenged on the day they were captured and then blood was sampled before animals were sacrificed.

Corresponding haematological and biochemical data will serve as reference values for wild-caught non-captive challenged individuals from High (H-LPS) and Low (L-LPS) polluted sites.

In experiment 2, a group of 36 individuals (23 captured in sites High, 13 from sites Low) was maintained in captivity for five days post-capture. Animals were blood sampled on the day they were captured (day 0, D0) without any treatment. Corresponding haematological and biochemical data will serve as reference values for wild-caught non-captive unchallenged individuals High (H) and Low (L) polluted sites, respectively (Table 1). Animals were immune-challenged at the end of the captivity period (day 5, D5) and were then sampled again for blood before sacrifice.

Animals held captive were fed with pellet corn starch food for rodents (SAFE® SAS - basis formula AIN93G, see content in Table S2). A first group of animals coming from High (H-LPS-Se+) and Low (L-LPS-Se+) polluted sites were fed the standard food while a second group coming from High (H-LPS-Se-) and Low (L-LPS-Se-) polluted sites were fed a Se-deficient food (Table S2). Vitamin E was also deprived from Se-deficient diet as vitamin E can compensate for the antioxidant activity and immune cell protective effect of Se (Noaman et al., 2002).

We acknowledge that laboratory studies investigating the effects of Se on the immune response of rodents have been done under a longer period (weeks)(e.g. El-Boshy *et al.*, 2015). However, a previous pilot study from our team showed that circulating (blood) levels of Se in wood mice fed standard rodent food changed after only five days of captivity (Powolny et al., In preparation). Moreover, laboratory rats fed with 0.5 mg Se/kg diet showed an increase of the glutathione peroxidase activity in only one day (Knight and Sunde, 1988). Furthermore, rats fed zinc-deficient diet for three weeks displayed a rapid (three to seven days) recovery of their immune function after a dietary zinc supplementation (Blewett and Taylor, 2012). A five-day lasting experiment may thus be expected as long enough to detect the early effects of Se on immune and inflammatory responses.

Housing

Captive animals (experiment 2) were housed individually (to avoid inter-individual aggressive interactions) in transparent boxes filled with a solid hemp litter, a shelter containing natural cotton and a paper roll as enrichment (Figure S2). Boxes were placed in shelves in a room with no heating system (i.e. day and night temperatures followed roughly the natural ones and was of 18-20°C during days and of 15-18°C at night for all the duration of the experiment). Light also followed nychthemeral regime. Animals had access to water and to food *ad libitum*. Individuals were weighed at day 0, and then at each cleaning of the box (i.e. at day 2, 4 and

5). Faecal samples and food remains were collected and weighed during each cleaning day. Faecal samples were stored at -20°C until analysis.

Blood and tissue sampling, immune challenge

Blood was sampled from the retro-orbital sinus (volume of 1% of the body mass) using a glass Pasteur pipette and was immediately transferred into EDTA-coated tubes (Microvette®, Sarstedt). A small volume of blood (~50µL) was used fresh (i.e. stored at +5°C until analysis, which was performed within 8h following sampling) for complete blood count. The rest of the blood (~150 µL) was centrifuged at 2000g for 15 minutes to collect plasma. Plasma was stored at -20°C during the field session and, when back at the laboratory, at -80°C until analyses.

Animals were challenged by intraperitoneal injection with a solution of bacterial lipopolysaccharides (LPS O111:B4, Merck) at 500 µg/kg, which is sufficient to enhance TNF- α production as found in several laboratory rodent models (Batista et al., 2019). Blood was collected two hours after LPS challenge.

After immune challenge and blood sampling, animals were sacrificed by cervical dislocation following ethical guidelines (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists, 2016). The liver and the eyes were extracted to measure trace metal concentrations in the liver and to determine age with crystalline lens mass (Vandorpe and Verhagen, 1979). Livers were stored at -20°C until further preparation.

Trace metal analysis

Livers were lyophilised until constant mass, then acid digested with nitric acid (HNO₃ 69%, ultra-pure quality for trace analyses) and then diluted with ultra-pure water (Elga, 18.2 MV/cm). The concentrations of Cd, Pb and Se in the liver were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Fisher Scientific X Series 2). The quality of measurement was assessed by regularly measuring calibration solutions and blank (ultra-pure water). Reproducibility was checked by using internal standards of the ICP-MS. Repeatability and accuracy were verified by repeated (6 times) measurements of certified reference materials (National Research Council Canada TORT-3 Lobster hepatopancreas, National Institute of Standards and Technology SRM® 1577c Bovine liver). Values under limits of quantification (LoQ) were replaced by the LoQ divided by the square root of two (Helsel, 2008). The concentration of metals was calculated in µg/g dry mass in the liver. Limits of quantification (LoQ), proportion of data < LoQ, certified values and average recoveries of the certified reference materials are provided in Table S3.

Complete blood count

Complete blood count was done with a Haematology Analyser automate (Horiba ABX Micros ES60), providing leukocyte counts (in 10^3 cells/mm³): white blood cells (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA). Repeatability is automatically assessed by the analyser by measuring 3 times each value. Moreover, each blood sample was analysed in 2 to 3 runs in the cases of non-repeatable measurements (Table S5). Reproducibility and accuracy were determined with daily blank controls and measurements of certified reference materials (MINOTROL 16, “low”, “medium” and “high”, Horiba SAS).

Determination of TNF- α

TNF- α plasma concentration was determined with a high sensitivity commercial Mouse TNF- α quantikine ELISA kit (R&D MH, detection range from 0.781 to 50 pg/ml). Positive controls were used to evaluate the ability of the kit to detect elevated values at 50 and 100 pg/ml (average recovery 86% in both cases). As low volume of blood did not allow performing linearity or other recovery assays, only two samples were used to control inter-assay (recovery of 104% and 105%) and two others sample to control intra-assay (recovery of 91% and 80%) quality of measurements. Four samples spiked with known amounts of TNF- α were used to assess if antigen-antibody inhibitors may be present in the sample, giving a recovery rate of 110% for a spike with 100 pg/ml ($n=1$), 92% with 25 pg/ml ($n=1$), and 89 and 73% with 3.13 pg/ml ($n=2$). Average recovery of 91% suggested the absence of such inhibitors.

Faecal and plasma CORT metabolite determination

The determination of faecal CORT metabolite (FCM) was performed according to the method of Touma *et al.* (2003) as detailed in Sánchez-González *et al.* (2018) on the wood mouse. Briefly, faecal samples were oven dried until constant mass (40°C for 10 hours) and homogenized with a mortar and a pestle. An aliquot of 0.056 ± 0.006 g of faeces was then mixed with 500 μ L of 80% methanol and 500 μ L of phosphate buffer, vortexed for 15 s and left 16 hours in an orbital agitator. Faecal extract of FCM was then centrifuged for 15 min at 2500 g and the supernatants were collected in microtubes and stored at -20 °C until analyses. FCM determination was done with a commercially available enzyme immunoassay rat/mouse serum/plasma kit from DEMEDITEC Diagnostics (Kiel, Germany). A serial dilution (1:32, 1:16, 1:8, 1:4, 1:2, 1:1) of faecal extract was done to evaluate parallelism of the standard curves and the diluted extracts (73 \pm 27%). Recovery assays were performed between plates to evaluate accuracy (63 \pm 24%). The CORT concentration expressed in ng/ml was then corrected by the individual faeces mass as it has been shown to have a significant influence on the levels of FCM (Navarro-Castilla and Barja, 2014). Plasma concentration of CORT was determined using the same DEMEDITEC Diagnostics kit. Assay precision was measured with intra-assay

and inter-assay recovery rates, which were respectively of $115\pm 47\%$ ($n=5$) and $90\pm 5\%$ ($n=4$). For accuracy, the recovery rate from spiked samples with 185 ng/ml of CORT was $99\pm 23\%$ ($n=2$) and the one from diluted (1:2) samples was $91\pm 26\%$ ($n=2$).

Ethics

Blood sampling, immune challenge and euthanasia were performed under gaseous anaesthesia using isoflurane (mobile anaesthesia unit UNIVENTOR 400). Experiments were performed under the authorization of the French National Ethical Committee (Project APAFIS N°5340) by skilled and experienced investigators from Chrono-environnement research department (EU0592), following directive 2010/63/EU on the protection of animals used for scientific purposes.

Statistical analysis

Two- or three-way ANOVA, Student *t*-test or Mann-Whitney test were performed to compare response quantitative variables (life-history trait parameters, metal and CORT concentrations...) among groups of explanatory variables (experiments, soil pollution levels, diet and LPS treatments...). *Post-hoc* analysis of Tukey's Honestly-Significant-Difference was done when interaction between group factors was significant. The 'HSD.test' function (package 'agricolae') was used as it considers unbalanced designs. Pairwise comparisons using paired *t*-tests with the method of Benjamini and Hochberg (1995) was performed when interaction between group variables was not significant and to compare repeated measurement of faecal CORT metabolites of each individual. Linear regressions or Spearman rank correlations were performed to test relationships between quantitative variables. \log_{10} transformation was applied to Cd and Pb concentrations, WBC, LYM, MON and GRA counts and CORT to satisfy prerequisite of parametric statistical tests (normality and homoscedasticity), which were verified with Mauchly, Kolmogorov-Smirnoff and Levene's tests (package 'performance'). All analyses were done with R version 3.6.1. (R Core Team, 2023) and graphics were drawn with 'ggplot2' and 'ggpubr' packages.

Results and discussion

Experiment 1: Effect of Cd and Pb on the immunocompetence of free-ranging wood mice

Variations of liver trace metal concentrations between pollution levels

Cd hepatic concentrations did not significantly differ between Low and High sites ($t = 1.2$, $df = 14.2$, $p = 0.24$, Table 2), while Pb concentrations were higher in mice from High sites ($t = 6.1$, $df = 13.8$, $p < 0.001$, Table 2). In previous studies conducted over the same area, a chronic exposure of free-living wood mice to both metals was observed but with significantly higher

internal organ concentrations in animals from the most polluted sites (Tête *et al.*, 2014). The contrasted findings between the two metals and between results of the different studies could be explained by variations in metal bioavailability. Indeed, comparing total concentrations and partitioning of metals in soil samples collected over the sites in 2006 and in 2019, Ozaki *et al.* (2022) showed changes in both exchangeable fractions and the less mobile fractions leading to an increase of Cd mobility in the less polluted site but a decreased mobility of Pb in the most contaminated sites.

Hepatic Se concentrations were higher in individuals from High than Low sites ($t = 4.4$, $df = 8.5$, $p = 0.002$, Table 2). A similar result was observed in a previous study over the same area (Powolny *et al.*, 2022). Peraza *et al.* (1998) hypothesised that Se, which is involved in a protective role against the toxicity of metals, would be preferentially taken up and/or accumulated in animals exposed to Cd and Pb. However, Se concentrations in soils differed between Low and High sites (median = 1.8 and 4.1 $\mu\text{g/g}$, respectively; Scheifler *et al.*, 2022). Therefore, our results did not allow discriminating between a physiologically “active” uptake or a “passive” Se accumulation because of higher soil Se concentrations in soils.

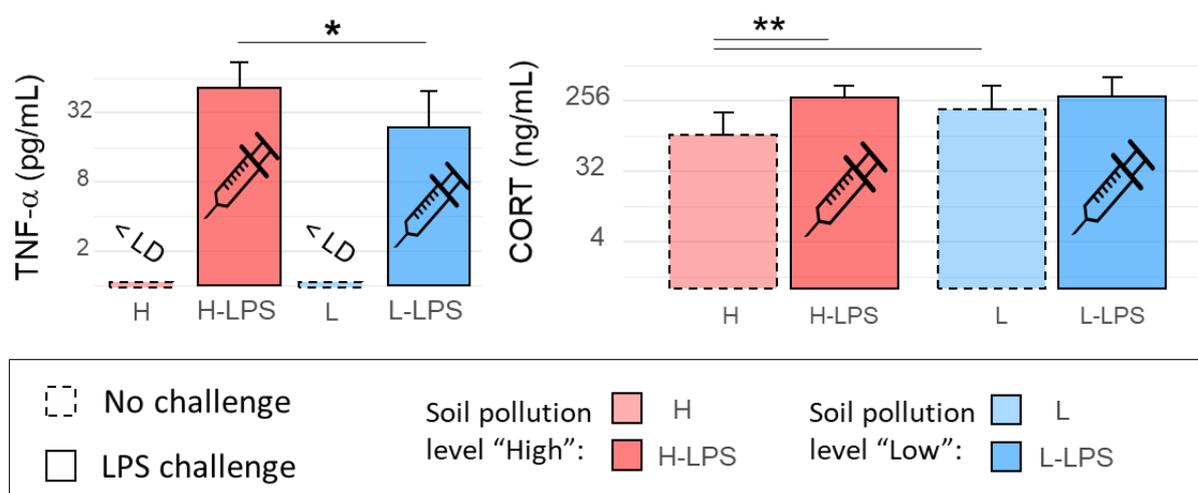
Table 2 Mean (and [median, min – max]) Cd, Pb and Se hepatic concentrations in the experimental groups. Different letters stand for significant differences between H-LPS and L-LPS.

Experiment	Group	Cd	Pb	Se
1	H-LPS	4.9 ^a [4.5, 0.9 - 11]	0.9 ^a [0.9, 0.6 - 1.6]	7.1 ^a [6.4, 5.1 - 10]
	L-LPS	4.8 ^a [1.3, 0.1 - 26]	0.2 ^b [0.04, 0.005 - 1.5]	2.8 ^b [2.5, 0.8 - 6]
2	H-LPS-Se+	8.0 ^a [4.0, 0.7 - 23]	1.1 ^a [0.6, 0.2 - 3.7]	6.8 ^a [6.7, 5.2 - 9.4]
	L-LPS-Se+	4.7 ^a [4.1, 0.2 - 16]	0.02 ^b [0.007, 0.006 - 0.08]	4.1 ^b [4.3, 3.4 - 5]
	H-LPS-Se-	5.1 ^a [0.9, 0.5 - 25]	1.2 ^a [0.5, 0.05 - 5.4]	5.4 ^c [5.5, 4.1 - 7.1]
	L-LPS-Se-	3.1 ^a [2.9, 0.3 - 5.8]	0.03 ^b [0.01, 0.006 - 0.1]	3.7 ^b [3.5, 2.6 - 5]

Influence of Cd and Pb exposure on the inflammatory response of free-ranging wood mice

All challenged wood mice exhibited an increase of TNF- α since its level was undetectable in animals before immune challenge (Table 3). This suggests that individuals displayed a functional response to the immune challenge, which at least produced the secretion of TNF- α . Concentrations of plasma TNF- α were found higher in H-LPS than in L-LPS individuals ($W = 53$, $p = 0.047$, Figure 1), suggesting a higher inflammatory response in animals from polluted sites (H-LPS).

Figure 1 Experiment 1. Concentration of plasma TNF- α (pg/ml) and of CORT (ng/ml) in response to LPS challenge in wood mice captured in contrasted levels (high and low) of soil pollution. Levels of significance of *t* and *U* (only TNF- α) tests, *, ** for $p < 0.05$, 0.01, respectively; <LD: below limits of detection.



Challenged individuals showed decreases of WBC count in comparison with unchallenged ones (Table 3, Figure S4). In individuals from High sites (H-LPS), leukocytes showed a significant decrease of -75% for WBC ($t = -5.7$, $df = 8.8$, $p < 0.001$), -77% for LYM ($t = -6.2$, $df = 10$, $p < 0.001$), -76% for MON ($t = -3.8$, $df = 6.7$, $p = 0.007$), and -51% for GRA ($t = -2.7$, $df = 8$, $p = 0.026$). In individuals from Low sites the variation of WBCs (-45 %; $t = 2.8$, $df = 23$, $p = 0.009$) may be related to LYM decrease (-52%; $t = 2.9$, $df = 22$, $p = 0.007$) as no variation was found for MON ($t = 1.7$, $df = 18$, $p = 0.09$) and GRA ($t = 2.1$, $df = 19$, $p = 0.05$). Similarly, Brooks *et al.* (2017) found that LPS (15 mg/Kg) induced a decrease of WBCs including LYM, GRA and MON within 4 hours post-injection in Wistar rats. The differences in leukocytes decrease between High and Low sites can be attributed to the elevated levels of TNF- α found in challenged animals from High sites. Negative relationships between WBC, LYM, MON and GRA and TNF- α concentrations were found (Figure S5).

Higher levels of TNF- α and a decrease of lymphocyte were evidenced in cell culture containing 100 ng/mL of LPS and rat peripheral blood polymorphonuclear leukocytes pre-treated with Cd (*i.p.* injection) at 1 mg/kg (Djokic *et al.*, 2014). Lead was also found to enhance a marked increase of TNF- α in rats after co-exposure with different concentrations of Pb and intravenous injection of LPS at 100 μ g/kg (Liu *et al.*, 2005). In our study, the difference of TNF- α concentrations and of circulating WBC levels between individuals from High and Low polluted

sites could suggest two levels of strength of the immune response to LPS challenge, with a possible peracute immune response in animals highly exposed to pollution.

Influence of Cd and Pb exposure on the anti-inflammatory response of free-ranging wood mice

Plasma CORT concentrations differed according to exposure to pollution and varied due to immune challenge in highly exposed individuals only. Lower levels of plasma CORT were found in unchallenged individuals from High sites in comparison with challenged ones from the same site and with unchallenged ones from the Low sites ($F = 5.2$, $df = 51$, $p = 0.003$, Figure 1). A significant increase of plasma CORT of 23% was found between challenged and unchallenged individuals from the High sites ($t = 5$, $df = 15$, $p < 0.001$; Figure 1 and Table 3) while no difference was found for animals from the Low sites ($t = 1$, $df = 22$, $p = 0.3$). Furthermore, WBC and LYM were negatively related to CORT but not MON and GRA (Figure S5). The absence of significant differences in GRA, MON and CORT in individuals from Low polluted sites could indicate a differential recruitment of WBCs. One explanation can be that, in highly exposed immune-challenged animals, CORT increased to avoid a hyper-inflammatory immune response. Indeed, one effect of CORT on the immune system of rodents is to control inflammation in modulating responses once infections are controlled to avoid self-damages mediated by the activated immune cells, also called autoimmunity (Medzhitov, 2008; Martin, 2009). Corticosterone can have down-regulating effects on immune parameters in reducing numbers of circulating LYM (T cells > B cells and CD4+ > CD8+) and also antigen presenting cells (*i.e.* macrophages, neutrophils and monocytes) promoting an immune-redistribution (Martin, 2009). In our study, the stress induced by the LPS challenge promoted a decrease of circulating LYM, and sometimes of other leukocytes, which may be mediated by elevated CORT enhancing a redistribution of WBCs from the blood to other organs where the infection occurs (*e.g.* peritoneal cavity, lymph node) and clear organisms from pathogens (Dhabhar, 2002). Lower levels of CORT in the plasma of free-ranging wood mice from the most polluted sites can be attributed to endocrine disruption mediated by chronic exposure to Cd and Pb. Indeed, Cd and Pb were found to inhibit circulating CORT by altering adrenal cortex functions (Nishiyama and Nakamura, 1984; Nishiyama et al., 1985). The adrenal cortex is mainly under the control of the pituitary adrenocorticotrophic hormone, which, by a negative feedback mechanism, controls plasmatic CORT levels (Wills and Havard, 2013). Lower circulating CORT could reduce anti-inflammatory levels in wood mice from polluted site. A dampening of anti-inflammatory levels would enable organisms exposed to pollution to maintain at a higher level their basal immune functions leading to an up-regulation of the immune system, as shown by the higher inflammatory response found in animals from the most polluted sites. One might hypothesize that a higher basal immune function in wood mice inhabiting polluted sites allows

a shortening of the time of effectiveness to enhance inflammatory immune response and thus quickly enhances CORT release in the blood stream.

Experiment 2: Effect of a Se-deficient diet on the immunocompetence of captive wood mice

Age structure

The age structure did not differ between individuals from the experiment 1 and 2 ($df = 47$, $F = 0.56$, $p = 0.57$) (Table S4). In both experiments 1 and 2, the concentrations of Cd, Pb and Se in the liver were not influenced by the age of individuals ($p > 0.05$).

Food consumption

Neither consumption ($df = 38$, $F = 0.32$, $p = 0.61$) nor body weight ($df = 47$, $F = 2.8$, $p = 0.07$) differed between individuals fed standard or Se-deficient food (Table S4), suggesting satisfactory housing and experimental conditions.

Variation of trace metals liver concentrations

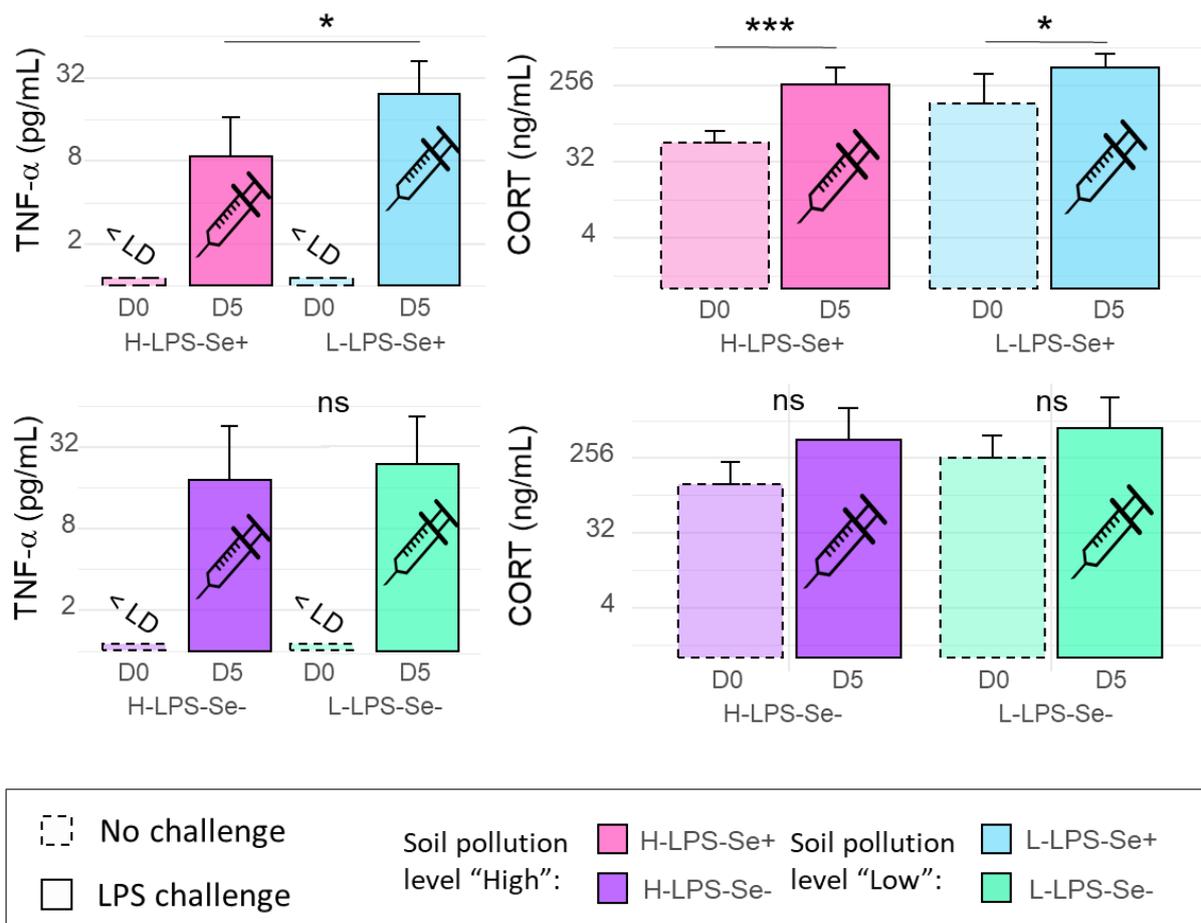
Neither Pb nor Cd concentrations in the liver of captive wood mice showed significant differences in comparison with those sampled the day they were captured (High: Pb: $df = 26$, $F = 0.68$, $p = 0.51$, Cd: $df = 26$, $F = 1.6$, $p = 0.21$; Low: Pb: $df = 23$, $F = 2.08$, $p = 0.15$; Cd: $df = 23$, $F = 0.09$, $p = 0.91$; Table 2). This absence of decrease in the hepatic Cd concentrations during captivity is in agreement with its very long half-life in rat and mouse (200-700 days) and in human (several years or decades) (Nordberg et al., 2015). Five days of captivity are not supposed to be long enough to detect a decrease in liver concentrations as its half-life in soft tissues is estimated at 40 days in human (Kabata-Pendias and Szteke, 2015). Individuals from High sites fed Se-deficient diet had lower Se concentration than those fed standard diet ($t = 3$, $df = 16$, $p = 0.01$, Table 2), while such a difference did not occur in individuals from Low sites ($t = 0.9$, $df = 8$, $p = 0.4$). Five days of captivity with Se-deficient food seemed sufficient to lead to a decrease of hepatic Se concentrations in wood mice exposed to metallic pollution. According to the literature, elimination of certain forms of Se in animals through both urine and faeces is at least biphasic, with one fast elimination phase of approximately one day (dose dependent) followed by a slower phase of 30-70 days in most of species (Alexander, 2015).

Influence of dietary Se on the inflammatory response of captive wood mice

After five days of captivity, all immune challenged wood mice displayed an increase of TNF- α since no detectable concentration were found in unchallenged individuals (Figure 2). As suggested earlier, the increase in the production of TNF- α might be interpreted as a functional response to LPS challenge promoting an inflammatory response (Brooks et al., 2017). Dietary Se induced differences in the pollution-mediated variations of TNF- α in challenged individuals. While no significant difference was found in TNF- α concentrations between individuals from

High and Low sites fed a Se-deficient diet ($p > 0.05$), TNF- α concentrations were lower in individuals from High than Low sites fed a standard diet ($W = 14, p = 0.03$, Figure 2).

Figure 2 Experiment 2. Concentration of plasma TNF- α (pg/ml) and of CORT (ng/ml) in response to LPS challenge in wood mice captured in contrasted levels (high and low) of soil pollution and fed for 5 days a standard or a Se-deficient diet. Levels of significance of t and U (only TNF- α) tests: ns: non-significant difference, *, *** for $p < 0.05, 0.01$, respectively; <LD: below limits of detection.



The concentrations of TNF- α did not differ between individuals from Low sites fed a standard versus a Se-deficient diet ($p > 0.05$). Individuals highly exposed to metals but fed with sufficient amount of Se (*i.e.* standard diet) may exhibit a lower inflammatory in response to LPS challenge than their counterparts lacking Se or not being stressed by toxic metals. However, the concentration of TNF- α did not display differences between individuals from High sites fed a Se-deficient diet in comparison with those fed a standard diet. Mammals' homeostasis as well as reproducing performances have been shown to be connected to individual micronutrient resources (Allen and Ullrey, 2004). Among them, Se is a key antioxidant which

deficiency could impact cardiac muscles (e.g. mulberry heart disease) and immune cells (i.e. leukocytes) functioning (Gombart et al., 2020). Selenium occurs in several mechanisms, including selenoproteins (e.g. GPX) and adducts (e.g. Cd-Se), which are involved in the detoxification of Cd and Pb (Fu et al., 2019; Vuksic et al., 2018). The critical role of Se in ROS scavenging activity was found to prevent immune cell damages (Avery and Hoffmann, 2018). Though pollution levels might have an effect on inflammatory response to immune challenge in animals fed a standard diet no difference was found when the TNF- α concentrations was compared between the 4 groups ($p > 0.05$, Table 3). Indeed, five days of captivity might be a short time to determine the effects of dietary Se-deficient diet on the inflammatory response to LPS in the wood mice.

At day 5, challenged wood mice from High sites fed a Se-deficient diet showed a significant decrease of LYM (-56%), while no significant changes was found in WBC, MON and GRA counts (Table 3, Figure S4). In contrast, challenged animals from High site but fed a standard diet displayed a significant decrease in WBC (-85%), LYM (-86%), MON (-87%) and no variation in GRA. Though no variation in TNF- α was found between H-LPS-Se+ and H-LPS-Se- the lower variation in leukocytes found in individuals fed a Se-deficient diet (i.e. only LYM decrease) might be interpreted as a lower inflammatory response in H-LPS-Se-. Similar contrasts between standard and Se-deficient diets were found for the Low sites since L-LPS-Se+ individuals showed a decrease in WBC (-53%), LYM (-56%), MON (-46%) and GRA (-71%) while only a reduction of WBC probably attributed to LYM decrease (-75%) was found in L-LPS-Se- (Table 3, Figure S4). These results might relate a potential influence of dietary Se on the inflammatory response as lower leukocyte variation was found in individuals fed a Se-deficient diet whilst no variation of the TNF- α was attributed to the diet (Table 3, Figure S4). Nonetheless, WBC and LYM were negatively related to TNF- α concentrations in individuals from High sites fed either a standard or deficient food (Figures 4 and S6, $r^2_{adj.} = 0.39$, $F = 4.8$, $df = 15$, $p = 0.015$). A significant interaction was found, showing a steeper negative relationships between LYM and TNF- α in individuals fed a standard diet than in those fed a Se-deficient diet (slope= -0.3 vs -0.02). In addition, such a pattern was not detected in animals from the Low sites. Altogether, these results might indicate that dietary Se has a positive influence on the immune response on wood mice exposed to high levels of Cd and Pb. El-Boshy *et al.* (2015) found in laboratory rats that dietary Se could reduce the inflammatory response (i.e; lower decrease of LYM and lower increase of TNF- α) induced by cadmium exposure. However, the functional role of dietary Se might be mitigated by inflammatory and anti-inflammatory processes which may last over months (Martin, 2009). These interpretations would therefore require further investigation.

Table 3 Variation of total leukocytes counts and corticosterone levels between unchallenged and challenged wood mice (for experiment 1: H vs H-LPS, L vs L-LPS and for experiment 2: D0 vs D5), and median (and [min-max]) concentrations of plasma TNF- α . NS stands for non-significant difference, *, **, *** for $p < 0.05$, 0.01, 0.001, respectively. Different letters stand for significant differences between H-LPS and L-LPS.

Experiment	Group	Δ^1 WBC	Δ LYM	Δ MON	Δ GRA	Δ CORT	TNF- α (pg/ml)	
1	H						< LD ²	
	H-LPS	-75% ***	-77% ***	-76% **	-51% *	+23% ***	49 ^a [3 - 94]	
	L						< LD	
	L-LPS	-45% *	-52% **	NS	NS	NS	24 ^b [0.7 - 80]	
2	H-LPS-Se+	D0					< LD	
		D5	-53% **	-56% *	-46% **	NS	+26% ***	4.5 ^a [0.05, 22]
	L-LPS-Se+	D0						< LD
		D5	-85% **	-86% **	-87% **	-71% *	+22% *	21 ^a [2, 44]
	H-LPS-Se-	D0						< LD
		D5	NS	-56% *	NS	NS	NS	11 ^a [1.8, 90]
	L-LPS-Se-	D0						< LD
		D5	-71% *	-75% **	NS	NS	NS	12 ^a [3.3, 73]

¹ Δ stands for variations of measured parameters (value of e.g. H-LPS - value of H)

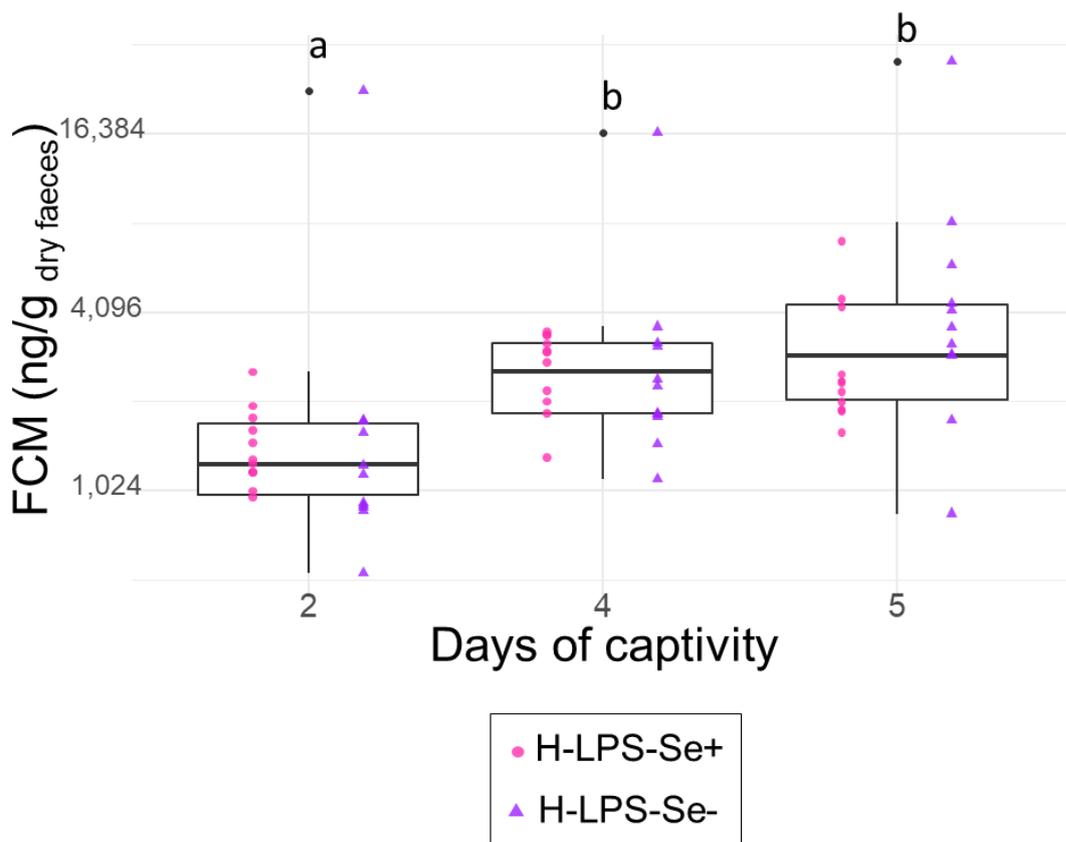
² < LD: under limits of detection of 0.295 pg/ml.

Influence of Se-deficient diet on the anti-inflammatory response of captive wood mice

Plasma CORT concentrations did not differ significantly between day 0 and day 5 in individuals from High and Low sites fed a Se-deficient diet (Table 3, Figure 2). Conversely, plasma CORT increased significantly by 26% and 22% between day 0 and day 5 in individuals from High and Low sites, respectively, fed a standard food (Table 3, Figure 2). FCM concentrations did not differ between individuals fed a standard or a Se-deficient diet ($F = 1.31$, $p = 0.26$, Figure 3) but varied according to pollution levels. FCM levels increased over the 5 days of captivity in individuals from High sites towards levels equivalent to the ones in animals from Low polluted sites (Figure 3). Lower FCM levels were found in captive individuals from High in comparison with Low sites after two days of captivity ($F = 17.1$, $p < 0.001$) while the concentrations did not differ at days 4 and 5. An increase of FCM in individuals from High sites from day 2 to 4 and from day 2 to 5, but not between days 4 to 5, was observed. No significant variations were

found between days 2, 4 and 5 in individuals from Low polluted sites. The mean FCM levels in individuals from High sites reaches the ones from Low at day 4. Interestingly, these results about FCM echoed and reinforced the results described above about lower plasma CORT concentrations in individuals from High than from Low sites.

Figure 3 Experiment 2. Trends in faecal corticosterone metabolite (FCM, ng/g_{dry faeces}) of wood mice captured in high level of soil pollution during five days of captivity. FCM was measured at day 2, 4 and 5 of the experiment in two groups of individuals fed a standard or a Se-deficient diet. Levels of significance of a pairwise paired T-tests between pollution levels: p-value > 0.05 (ns), <0.05 (*), <0.01 (**), <0.001(***)).



These findings might be interpreted as a functional response of CORT to dietary Se where individuals fed with sufficient amount of Se (*i.e.* standard diet) produced higher anti-inflammatory CORT levels. As discussed previously, the increase of CORT could promote a redistribution of WBCs from the blood to the sites of infection (Dhabhar, 2002).

Significant variations of CORT and leukocyte counts occurred in individuals fed a standard diet but not in the ones fed a Se-deficient diet (Table 4). Negative relationships were found between LYM counts and CORT concentrations in individuals from High sites ($r^2_{adj} = 0.53$, $F = 7.1$, $df = 13$, $p = 0.005$) with a lower slope in individuals fed a Se-deficient diet than in individuals fed a standard diet (slope= -1.1 vs -0.3, Figure 4).

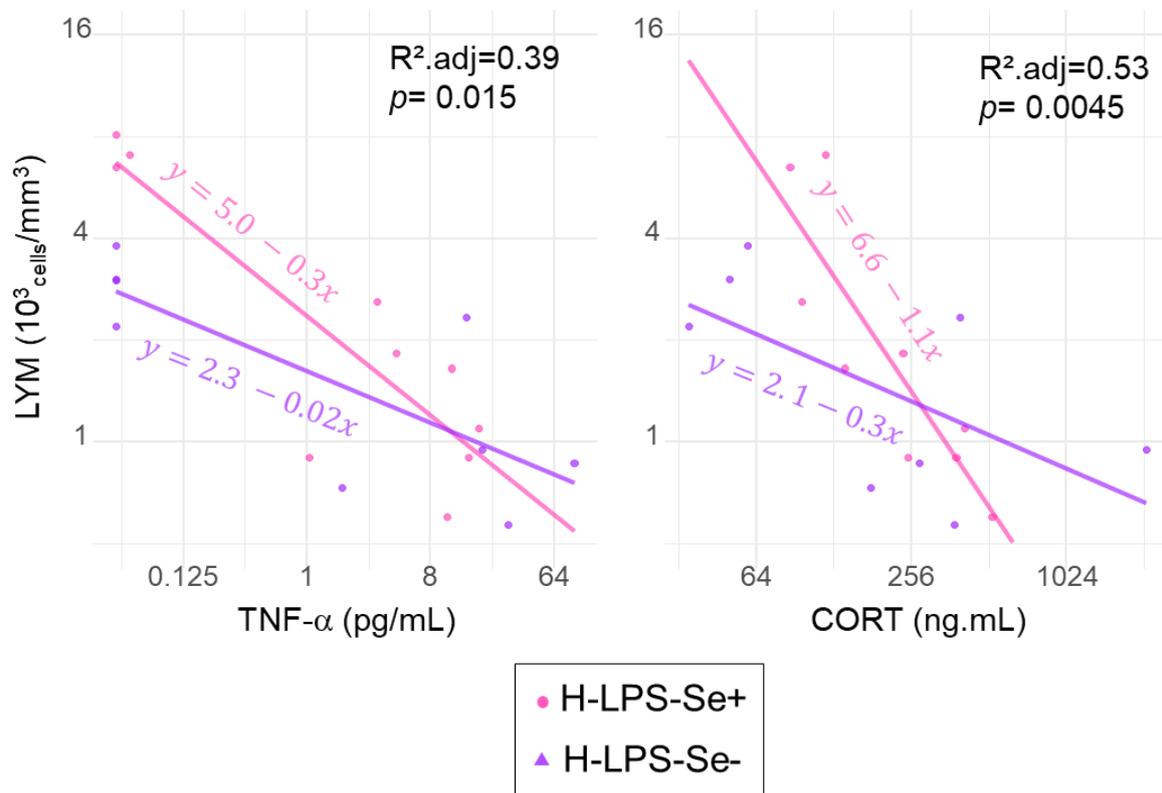
Table 4 Summary of the main trends in the experiments, in terms of stressors encountered by animals and responses as measured by variation of WBCs, CORT, and TNF- α and by Cd, Pb, and Se concentrations. Only statistically significant variations are represented. Decreases and increases are represented by \searrow and \nearrow , respectively. Variations of 0 to 33%, 34 to 66%, and 67 to 100% are represented by 1, 2 and 3 arrows, respectively. Cd, Pb, and Se concentrations are qualified of high (H) or low (L), and an arrow is used to represent a decrease/increase during captivity.

Group	Stressors	WBCs	LYM	MON	GRA	CORT	TNF- α	Cd	Pb	Se
H-LPS	High exposure	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$	\nearrow	$\nearrow\nearrow\nearrow$	H	H	H
	Natural conditions									
L-LPS	Low exposure	$\searrow\searrow$	$\searrow\searrow$				$\nearrow\nearrow$	H	L	L
	Natural conditions									
H-LPS-Se+	Past high exposure									
	Captivity (no exposure) Standard food	$\searrow\searrow$	$\searrow\searrow$	$\searrow\searrow$		$\nearrow\nearrow$	\nearrow	H	H	H
L-LPS-Se+	Past low exposure									
	Captivity (no exposure) Standard food	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$	$\nearrow\nearrow$	$\nearrow\nearrow$	H	L	L
H-LPS-Se-	Past high exposure									
	Captivity (no exposure) Se-deficient food		$\searrow\searrow$				\nearrow	H	H	H \searrow
L-LPS-Se-	Past low exposure									
	Captivity (no exposure) Se-deficient food	$\searrow\searrow\searrow$	$\searrow\searrow\searrow$				\nearrow	H	L	L

Dietary Se might counteract Cd and/or Pb impacts on the immune system allowing individuals fed a standard diet to perform an efficient immune response. As discussed above, lower CORT levels in individuals from High sites might be attributed to endocrine disturbance induced by Cd or Pb exposure (Nishiyama and Nakamura, 1984; Nishiyama et al., 1985). The variations of CORT levels may provide an explanation of the discrepancies found in TNF- α concentrations, which may be lower in individuals from High polluted sites fed a standard diet, as higher basal CORT levels could promote a higher anti-inflammatory activity. In contrast, individuals fed a Se-deficient diet showed a lower immune response to the immune challenge as LYM could probably be less dependent to plasmatic TNF- α and CORT. Lower quality of

diet regarding Se would thus disturb both pro- and anti-inflammatory activity of TNF- α and CORT in individuals exposed to toxic metals.

Figure 4 Experiment 2. Relationships between lymphocyte counts and plasma TNF- α and CORT after LPS challenge in captive wood mice from “High” levels of soil pollution fed a standard (H-LPS-Se+) or a Se-deficient (H-LPS-Se-) diet.



Conclusion

Our study provides new insight at the interface of immunology and ecotoxicology to highlight physiological disturbances originating from anthropogenic activities in a terrestrial small mammal. Individuals caught in polluted sites showed higher exposure to and accumulation of Pb and Se, a higher exposure to but not accumulation of Cd, and a significant decrease in hepatic concentrations during captivity for Se only. Free-ranging animal chronically exposed to Cd and Pb displayed a higher inflammatory response to immune challenge with a decrease in leukocytes (mainly mediated by lymphocytes) and a higher production of TNF- α and CORT. The increase in the immunocompetence of individuals from the polluted site was attributed to neuroendocrine disturbance which might reduce the production of anti-inflammatory CORT. Wild-caught animals maintained in captivity fed a Se-deficient diet showed a lower immune response to LPS challenge than ones fed a standard diet, which might be explained by a

lowered immune function in captive individuals fed a low quality diet. Altogether, our results highlight an immunomodulation of the inflammatory response indirectly mediated by Cd and/or Pb exposure and diet quality, related to a disturbance in circulating CORT levels. The role of dietary Se on the inflammatory and anti-inflammatory responses to challenge remains unclear. Further experiments on longer period of captivity considering the nutritional status of individuals might allow determining how dietary Se could display a functional role on the immunocompetence of wild animals chronically exposed to Cd and Pb.

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Data availability

Data will be made available on request.

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Supplementary Material

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Table S1 Characteristics of the study sites (made of two replicate sites for each level of pollution, distance from the former smelter, median, minimum and maximum soil concentrations of Cd, Pb and Se with year of sampling and analysis in brackets) near the former Metaleurop Nord smelter.

		High	Low
Distance from Metaleurop Nord (median, km)		0.6 (two adjacent sites)	3.0-11.0 (one at 3, the other at 11 km from the former smelter)
Soil metal concentrations ($\mu\text{g/g}$ dry mass)	Cd (2006)	59.3 (15.3 – 236.5)	1.4 (0.7 – 5.3)
	Pb (2006)	1357 (755 – 6809)	116 (43 – 200)
	Se (2019)	4.1 (3.1 – 10.0)	1.8 (1.5 – 2.3)

Table S2 Composition of standard (AIN93G) and Se-deficient (AIN Deficient) food provided to wood mice for 5 days in captivity (average quantity of 10.1 ± 0.2 g of pellets given every two days).

		AIN93G 18% casein	AIN DEFICIENT Selenium and Vit.E tocophe
mg / kg	Selenium (Se)	0.44	< 0,1
UI / kg	Vit. E (dl-alpha-tocopherol acetate)	87.4	6.9
%	Nitrogen-free extract	63.1	61.6
%	Starch	47.9	48.5
%	Total sugars	13.8	12.2
%	Crude Protein	15.9	16.1
%	Crude Fat	7.4	7.4
%	Crude Ash	2.7	2.7
%	Crude fiber	3.6	3.6
%	Humidity	7.3	8.6
Kcal / kg	ME Atwater Kcal	3,827	3,773
%	% Energy from proteins	16.7	17.1
%	% Energy from lipids	17.4	17.6
%	% Energy from Nitrogen-free extract	65.9	65.3
%	% Energy from sugar	14.5	12.9
%	% Energy from starch	50.1	51.5
mg / kg	Calcium (Ca)	4,973	5,013
mg / kg	Phosphorus (P)	2,621	2,657
mg / kg	Sodium (Na)	1,437	1,582
mg / kg	Zinc (Zn)	42.5	43.8
UI / kg	Vit. A	5,237	4,501
UI / kg	Vit. D3	1,250	1,250

Table S3 Limits of quantification (LoQ) of ICP-MS analysis of metals, proportion of data < LoQ, certified values and average recoveries of the certified reference materials used in this study (the number of reference samples analyzed for each certified reference material is 6).

Parameter	Cd	Pb	Se
LoQ (µg/L)	0.018	0.006	0.056
Proportion of data under LoQ (%)	High: 0% Low: 0%	High: 0% Low: 38%	High: 0% Low: 0%
TORT-3 certified values (and uncertainty: 95 confidence limits) in µg/g	42.3(1.8)	0.225(0.018)	10.9(1.0)
Recovery (%)	93±2	71±1	99±3
SRM 1577c certified values (and uncertainty: 95 confidence limits) in µg/g	0.097(0.001)	0.063(0.001)	2.031(0.045)
Recovery (%)	70±5	61±7	105±3

Table S4 Age, whole body mass and food consumption (median, [min - max]) of individuals in function of group (with sample size in brackets) and experiment.

Group	Experiment	Age at sacrifice (in days)	Whole body mass (in g)				Daily quantity of food consumed (in g)		
			D0	D2	D4	D5	D0-D2	D2-D4	D4-D5
H-LPS (6)	1	200 [58 - 260]	22.9 [14.9 - 25.7]						
L-LPS (13)	1	105 [47 - 205]	18.5 [14.6 - 21.7]						
H-LPS-Se+ (12)	2	130 [56 - 486]	17.6 [14.8 - 23.7]	18.1 [15 - 24.3]	17.5 [14.1 - 25.1]	17.5 [14.4 - 24.8]	2.8 [2.5 - 4.4]	3.2 [2.3 - 4.5]	3.1 [0.4 - 4.9]
L-LPS-Se+ (7)	2	108 [53 - 251]	19.3 [15.3 - 23.4]	18.4 [16.6 - 25.2]	19.6 [16.9 - 24.7]	19.1 [15.7 - 23.7]	3.6 [2.6 - 4.1]	3.8 [3.0 - 3.9]	3.4 [0.8 - 4.2]
H-LPS-Se- (11)	2	116 [58 - 502]	16.4 [15.6 - 22.7]	17.5 [15.8 - 21.6]	18.0 [15.9 - 21.3]	17.5 [16.4 - 20.9]	3.2 [2.6 - 3.9]	3.5 [3.1 - 4.5]	3.2 [2.5 - 3.8]
L-LPS-Se- (6)	2	99 [48 - 264]	15.9 [15.1 - 22.6]	16.0 [14.9 - 20.3]	16.5 [14.9 - 20.8]	16.4 [14.6 - 20.8]	3.2 [2.4 - 3.9]	3.6 [3.2 - 4.4]	3.7 [3.1 - 4.7]

Table S5 Raw data of blood counts given for each individual captured during the field session. ID: individual code; EXP: experiment 1 or 2; DAY: day 0 or 5 of experiment 2, or CS: free-ranging animals (not maintained in captivity); POLL: pollution level; WBC: White blood cells; LYM: Lymphocytes, MON: Monocytes; Granulocytes.

ID	EXP	DAY	POLL	WBC (10 ³ /mm ³)	LYM (10 ³ /mm ³)	MON (10 ³ /mm ³)	GRA (10 ³ /mm ³)
D01	2	0	Low	9.4	8.3	0.87	0.30
				9.3	8.3	0.80	0.20
				9.5	8.4	0.80	0.30
				9.5	8.1	1.00	0.40
D01	2	5	Low	0.2	0.1	0.00	0.10
D02	2	0	Low	2.5	1.8	0.40	0.30
D02	2	5	Low	1.0	0.5	0.20	0.30
F01	2	0	Low	6.3	4.3	1.10	0.90
F01	2	5	Low	1.1	0.8	0.10	0.17
				1.1	0.9	0.10	0.10
				1.1	0.8	0.10	0.20
H01	2	0	Low	3.2	2.6	0.40	0.20
H01	2	5	Low	0.2	0.1	0.00	0.10
				0.2	0.1	0.00	0.10
				0.2	0.1	0.00	0.10
H02	2	0	Low	3.1	2.5	0.40	0.20
H02	2	5	Low	1.7	0.8	0.50	0.40
J01	2	0	Low	11.6	8.4	2.30	0.90
				11.5	8.4	2.30	0.80
				11.6	8.3	2.30	1.00
J01	2	5	Low	2.1	1.5	0.30	0.35
				2.1	1.5	0.30	0.30
				2.1	1.4	0.30	0.40
L01	2	0	Low	8.9	6.6	1.47	0.83
				8.9	7.3	1.30	0.30
				9.1	7.3	1.30	0.50
				8.6	5.1	1.80	1.70
L01	2	5	Low	1.1	0.7	0.15	0.25
				1.1	0.7	0.20	0.20
				1.0	0.6	0.10	0.30
N01	1	CS	Low	2.6	2.4	0.10	0.15
				2.6	2.4	0.10	0.10
				2.6	2.3	0.10	0.20
N02	1	CS	Low	2.0	1.4	0.20	0.35
				1.9	1.4	0.20	0.30
				2.0	1.4	0.20	0.40
F01	2	0	High	2.9	2.1	0.55	0.25
				2.8	2.0	0.60	0.20
				2.9	2.1	0.50	0.30

F01	2	5	High	1.1	0.9	0.03	0.17
				1.1	0.9	0.10	0.10
				1.1	0.9	0.00	0.20
G01	2	0	High	3.9	2.3	0.75	0.85
				3.8	2.3	0.80	0.70
				4.0	2.3	0.70	1.00
G01	2	5	High	1.7			
G02	2	0	High	19.3	17.1	1.60	0.53
				18.7	16.6	1.60	0.50
				20.5	18.3	1.60	0.60
G02	2	5	High	3.2	2.3	0.53	0.33
				3.2	2.4	0.50	0.30
				3.3	2.3	0.60	0.40
H01	2	0	High	3.9	2.7	0.60	0.67
				3.9	2.7	0.60	0.60
				4.0	2.7	0.60	0.70
I01	2	0	High	9.3	7.2	1.50	0.60
				9.3	7.2	1.50	0.60
I01	2	5	High	1.5	1.1	0.20	0.20
J01	2	0	High	6.3	5.1	0.80	0.40
J01	2	5	High	1.3	1.0	0.20	0.10
A01	2	0	High	10.5	8.0	1.70	0.80
A01	2	5	High	10.7	8.1	1.60	1.00
C02	2	0	High	1.6	1.2	0.20	0.20
C02	2	5	High	9.8	7.8	1.10	0.90
C03	2	0	High	11.3	8.5	2.07	0.73
				11.0	8.4	2.00	0.60
				11.4	8.6	2.10	0.70
				11.5	8.5	2.10	0.90
C03	2	5	High	5.9	3.6	1.40	0.90
C04	2	0	High	3.4	3.0	0.20	0.25
				3.3	2.9	0.20	0.20
				3.5	3.0	0.20	0.30
C04	2	5	High	4.9	3.0	1.00	0.85
				4.8	3.0	1.10	0.70
				4.9	3.0	0.90	1.00
D01	2	0	High	8.9	7.6	1.00	0.35
				8.7	7.5	0.90	0.30
				9.1	7.6	1.10	0.40
D01	2	5	High	0.6	0.5	0.00	0.10
D02	2	0	High	4.5	3.2	0.73	0.50
D02	2	5	High	2.0	1.7	0.10	0.20
D03	2	0	High	8.8	6.8	1.15	0.85
				8.6	6.7	1.10	0.80
				8.9	6.8	1.20	0.90

D03	2	5	High	67.4	42.2	9.80	15.35
				66.9	41.8	9.80	15.30
				67.8	42.6	9.80	15.40
D04	2	0	High	19.7	16.1	1.90	1.75
				20.0	16.5	1.80	1.70
				19.4	15.6	2.00	1.80
D04	2	5	High	1.2	0.9	0.10	0.20
E02	2	0	High	4.7	3.8	0.50	0.40
E02	2	5	High	4.5	2.2	1.45	0.80
E03	2	0	High	5.0	4.6	0.30	0.15
				4.9	4.5	0.30	0.10
				5.1	4.6	0.30	0.20
E04	2	0	High	8.7	7.3	1.00	0.40
E04	2	5	High	0.9	0.6	0.10	0.20
F01	2	0	High	9.1	7.6	1.05	0.50
F01	2	5	High	9.6	6.6	2.17	0.87
				9.4	6.5	2.10	0.80
				10.0	6.7	2.30	1.00
F02	2	0	High	2.8	2.5	0.20	0.10
F02	2	5	High	0.9	0.6	0.10	0.20
G02	1	CS	High	4.2	2.8	0.93	0.53
				4.2	2.8	0.95	0.50
				4.3	2.8	0.90	0.60
H01	2	0	High	10.1	8.9	0.90	0.35
				9.8	8.7	0.80	0.30
				10.4	9.0	1.00	0.40
H01	2	5	High	2.2	1.8	0.17	0.20
H02	1	CS	High	1.4	1.0	0.15	0.20
I01	2	0	High	5.6	4.4	0.80	0.40
I01	2	5	High	1.0	0.7	0.10	0.20
I03	2	0	High	3.9	3.4	0.30	0.20
				3.8	3.4	0.30	0.10
				3.9	3.3	0.30	0.30
I03	2	5	High	3.1	2.6	0.30	0.17
				3.0	2.6	0.30	0.10
				3.1	2.6	0.30	0.20
J01	2	0	High	6.7	5.9	0.40	0.40
J01	2	5	High	4.2	3.1	0.65	0.45
				4.3	3.2	0.70	0.40
				4.1	3.0	0.60	0.50
J03	2	0	High	12.1	7.6	3.65	0.85
				11.8	7.4	3.60	0.80
				12.4	7.8	3.70	0.90
J03	2	5	High	10.1	7.1	2.30	0.70
				9.7	6.7	2.40	0.60

				10.2	7.3	2.20	0.70
				10.3	7.2	2.30	0.80
J05	2	0	High	4.0	3.1	0.67	0.30
J05	2	5	High	1.3	0.9	0.10	0.30
				1.1	0.8	0.10	0.20
				1.1	0.7	0.10	0.30
				1.6	1.1	0.10	0.40
K01	1	CS	High	1.0	0.8	0.00	0.18
				1.0	0.9	0.00	0.10
				1.0	0.8	0.00	0.20
K02	1	CS	High	1.5	1.1	0.20	0.20
K04	1	CS	High	1.9	1.5	0.10	0.25
				1.7	1.4	0.10	0.20
				2.0	1.6	0.10	0.30
L01	1	CS	High	1.8	1.6	0.10	0.10
A01	2	0	Low	6.5	5.2	0.80	0.55
				6.4	5.1	0.80	0.50
				6.6	5.2	0.80	0.60
A01	2	5	Low	1.8	1.4	0.10	0.30
I01	2	0	Low	2.9	2.2	0.40	0.25
				2.9	2.3	0.40	0.20
				2.8	2.1	0.40	0.30
I01	2	5	Low	2.2	1.5	0.40	0.35
				2.1	1.4	0.40	0.30
				2.2	1.5	0.40	0.40
I02	1	CS	Low	4.8	2.8	1.00	0.95
				4.6	2.7	1.00	0.90
				4.9	2.9	1.00	1.00
I02	2	0	Low	2.8	2.1	0.40	0.35
				2.8	2.1	0.40	0.30
				2.8	2.0	0.40	0.40
I02	2	5	Low	1.5	1.0	0.20	0.25
				1.4	1.0	0.20	0.20
				1.5	1.0	0.20	0.30
L02	1	CS	Low	1.0	0.7	0.10	0.20
				1.0	0.7	0.10	0.20
L05	1	CS	Low	5.1	3.8	0.95	0.35
				5.1	3.8	1.00	0.30
				5.0	3.7	0.90	0.40
B01	1	CS	Low	0.7	0.6	0.00	0.10
B02	1	CS	Low	1.3	0.8	0.10	0.40
N01	2	5	Low	1.0	0.7	0.10	0.20
NO							
1	2	0	Low	9.5	7.5	1.40	0.57
				9.3	7.4	1.40	0.50
				9.6	7.6	1.40	0.60

O01	2	0	Low	7.1	5.0	0.90	1.20
O01	2	5	Low	0.4	0.2	0.00	0.18
				0.4	0.3	0.00	0.10
				0.4	0.2	0.00	0.20
R01	1	CS	Low	9.5	5.2	2.60	1.63
				9.0	5.4	2.40	1.20
				9.3	4.9	2.70	1.70
				10.1	5.4	2.70	2.00
S01	2	0	Low	8.7	5.1	1.80	1.80
S01	2	5	Low	1.4	1.1	0.20	0.13
				1.4	1.1	0.20	0.10
				1.5	1.1	0.20	0.20
S02	1	CS	Low	1.3	0.9	0.20	0.20
S03	1	CS	Low	2.1	1.5	0.15	0.45
				2.1	1.5	0.20	0.40
				2.1	1.5	0.10	0.50
S04	1	CS	Low	7.9	6.0	1.37	0.57
				7.6	5.8	1.30	0.50
				8.1	6.1	1.40	0.60
T01	1	CS	Low	1.3	0.8	0.10	0.40
T02	1	CS	Low	5.8	2.9	2.17	0.80
				6.0	3.0	2.30	0.70
				5.7	2.8	2.10	0.80
				5.8	2.8	2.10	0.90

Figure S1 Experimental design.

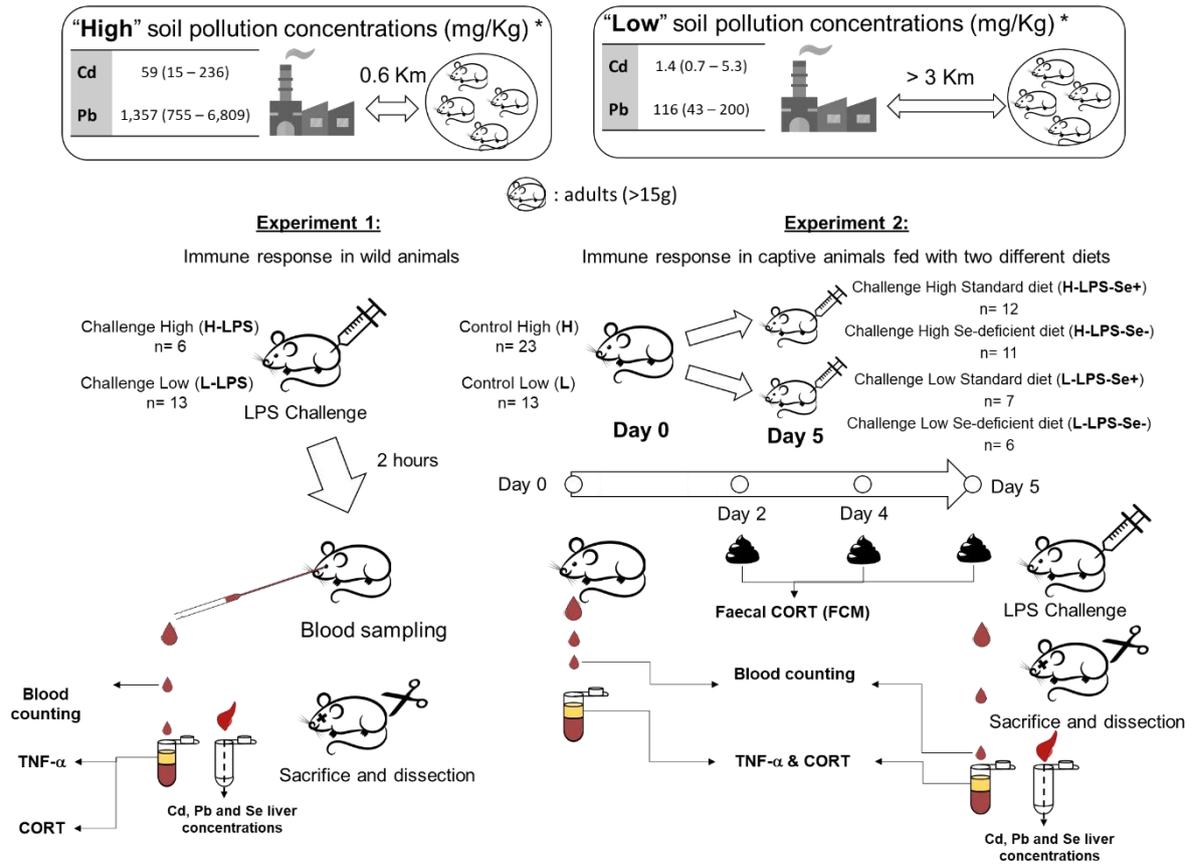


Figure S2 Picture of boxes where animals were maintained in captivity for 5 days.



Figure S3 Cadmium (Cd), lead (Pb) and selenium (Se) concentrations ($\mu\text{g/g}$, log scaled) in the liver of captive and non-captive wood mice among contrasted levels (high and low) of pollution. Levels of significance: $p > 0.05$: ns, $p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***.

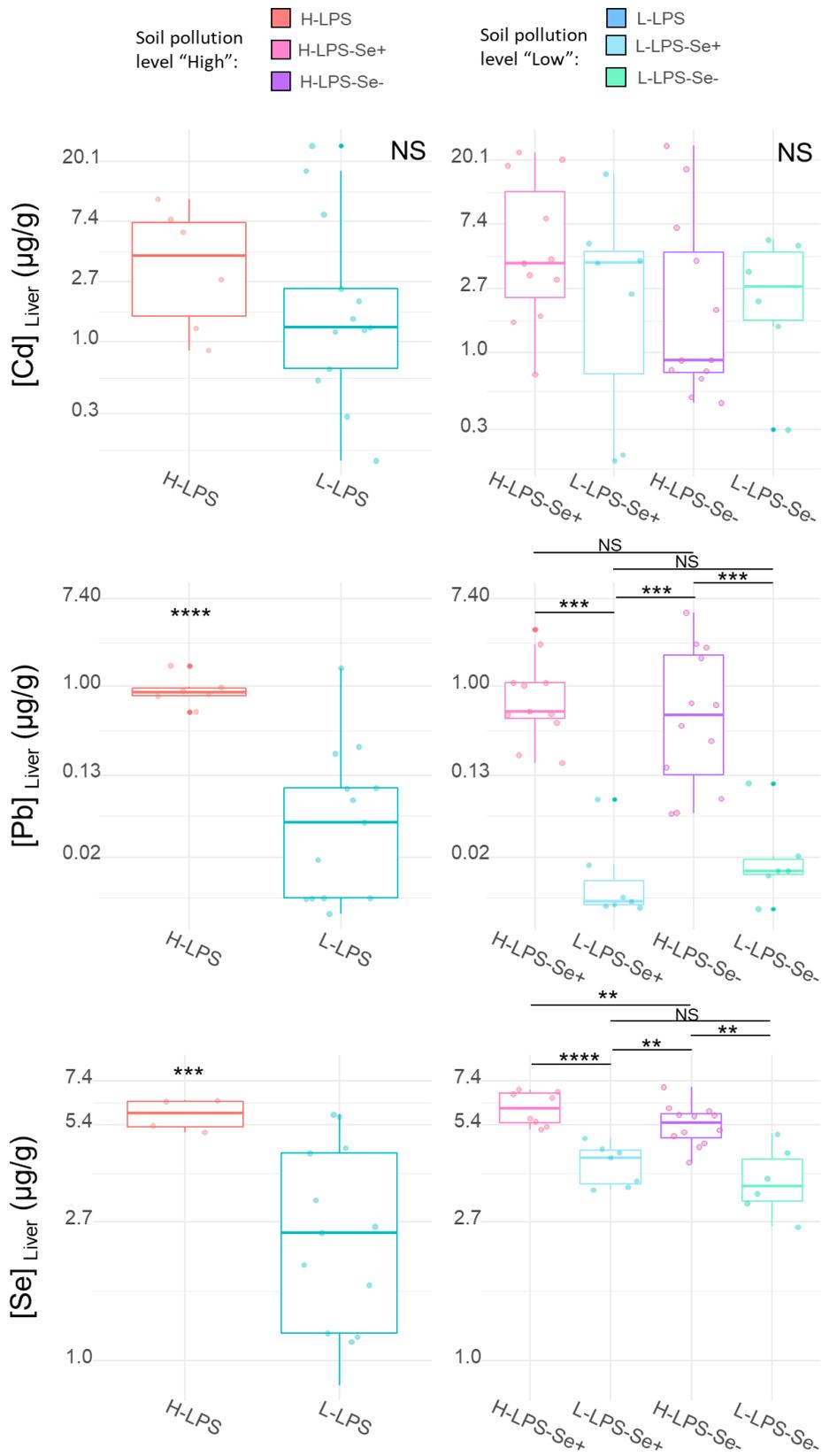


Figure S4 Leukocytes (WBC, log scaled) count between challenged (H- or L-LPS, H- or L-LPS-Se+ D5, H- or L-LPS-Se- D5) and unchallenged (H, L, H- or L-LPS-Se+ D0, H- or L-LPS-Se- D0) wood mice from contrasted levels of soil pollution with Cd and Pb (see Tables 1 and S1 for acronyms). Levels of significance: $p > 0.05$: ns, $p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***.

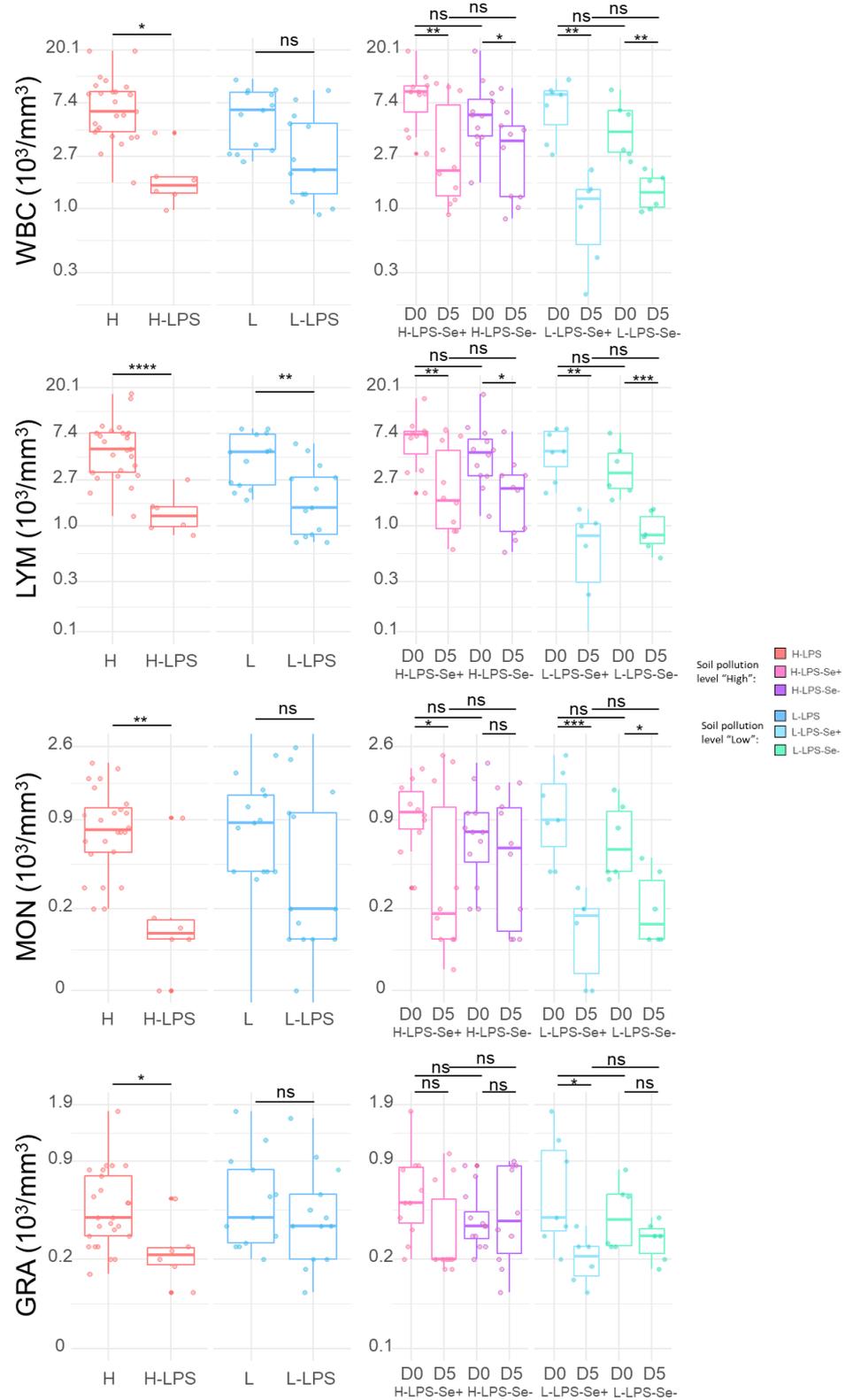


Figure S5 Experiment 1. Variations of white blood cells (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA) counts in response to plasma TNF- α and CORT after LPS challenge in individuals from “High” (red dots) and “Low” (blue dots) levels of soil pollution.

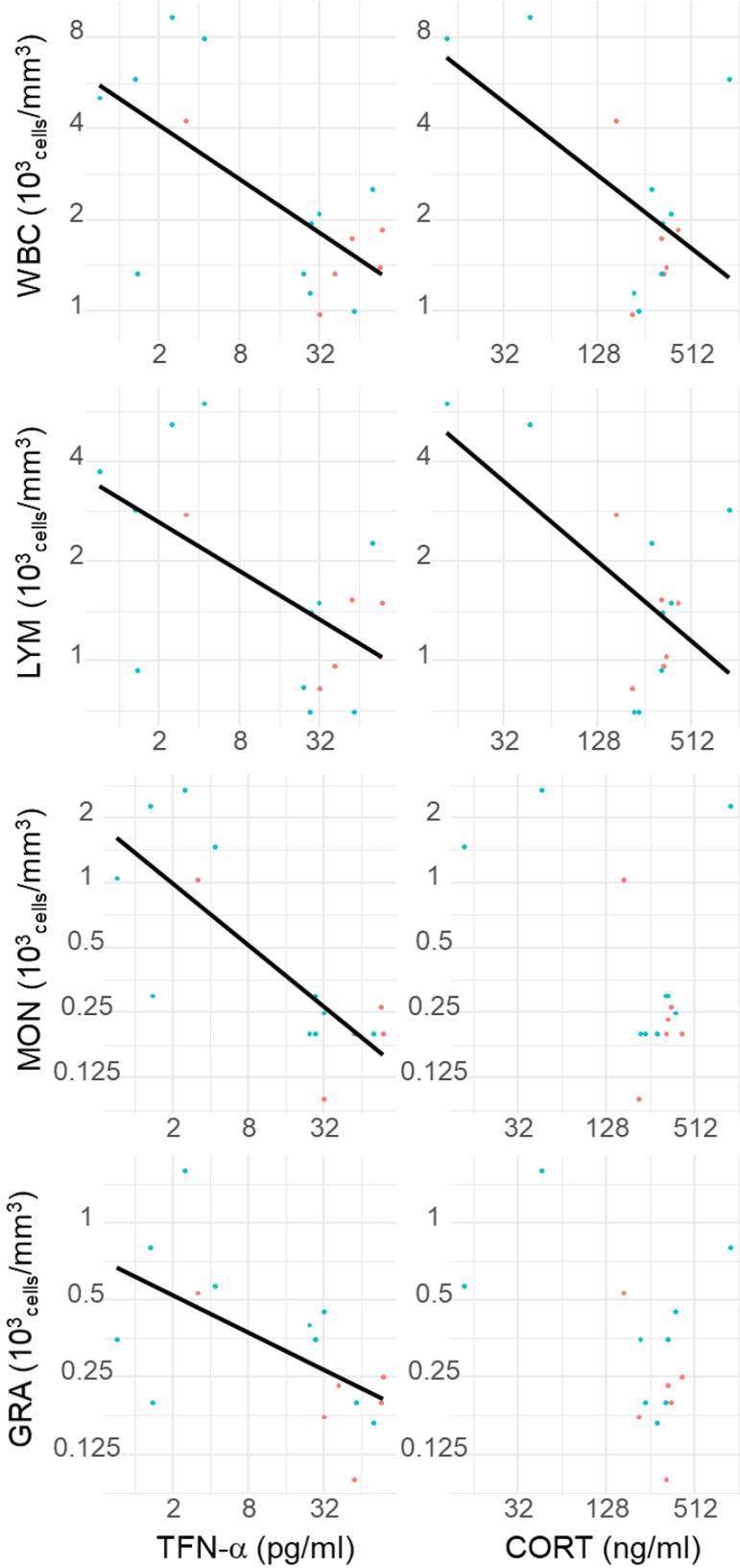


Figure S6 Experiment 2. Variations of white blood cells (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA) counts in response to plasma TNF- α and CORT after LPS challenge in captive wood mice from “High” levels of soil pollution either fed with standard (H-LPS-Se+) and Se-deficient (H-LPS-Se-) diets.

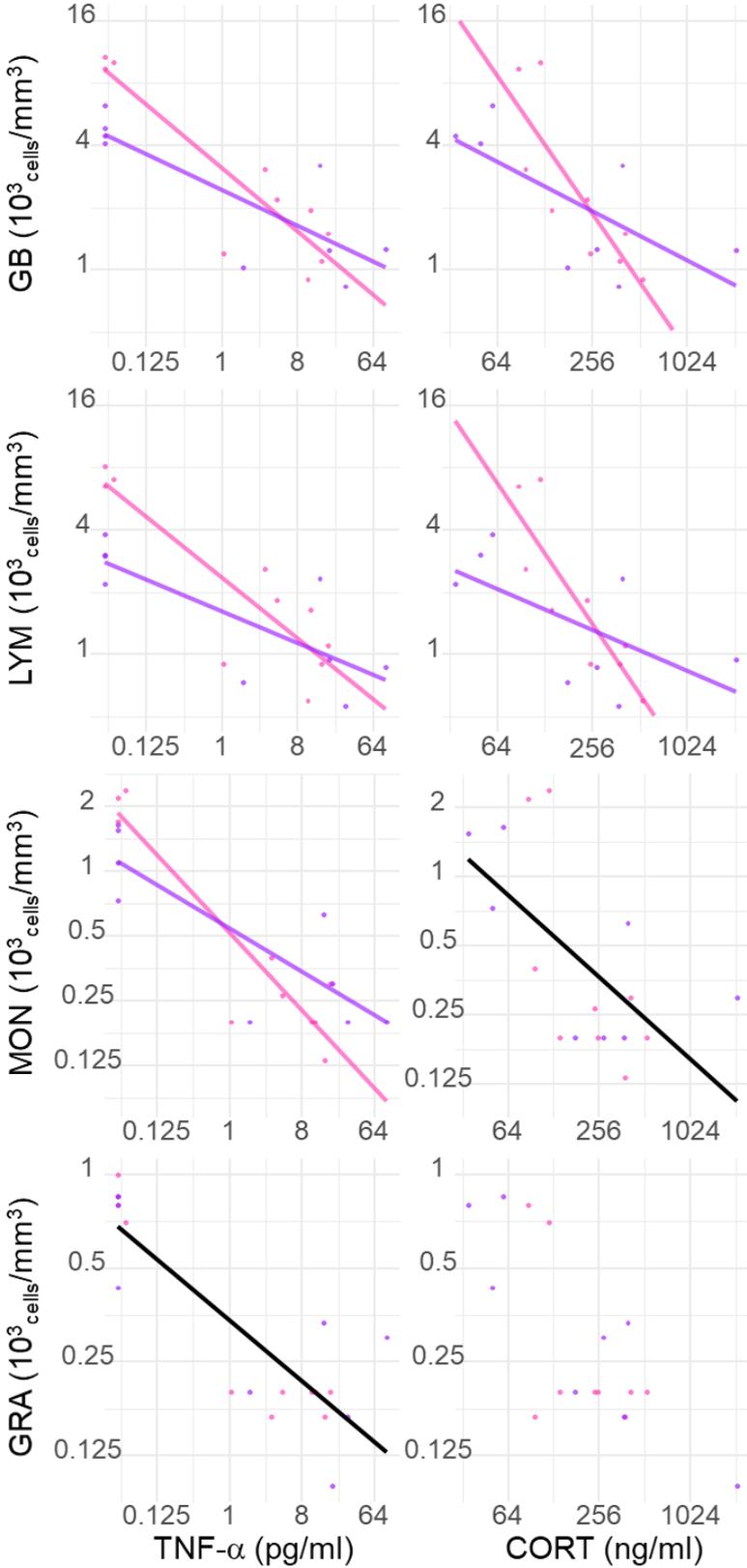


Figure S7 Correlation matrix of Spearman of the physiological variables measured in wood mice captured on contrasted levels of pollution (high and low) measured 2 hours after LPS challenge (i.p. injection).

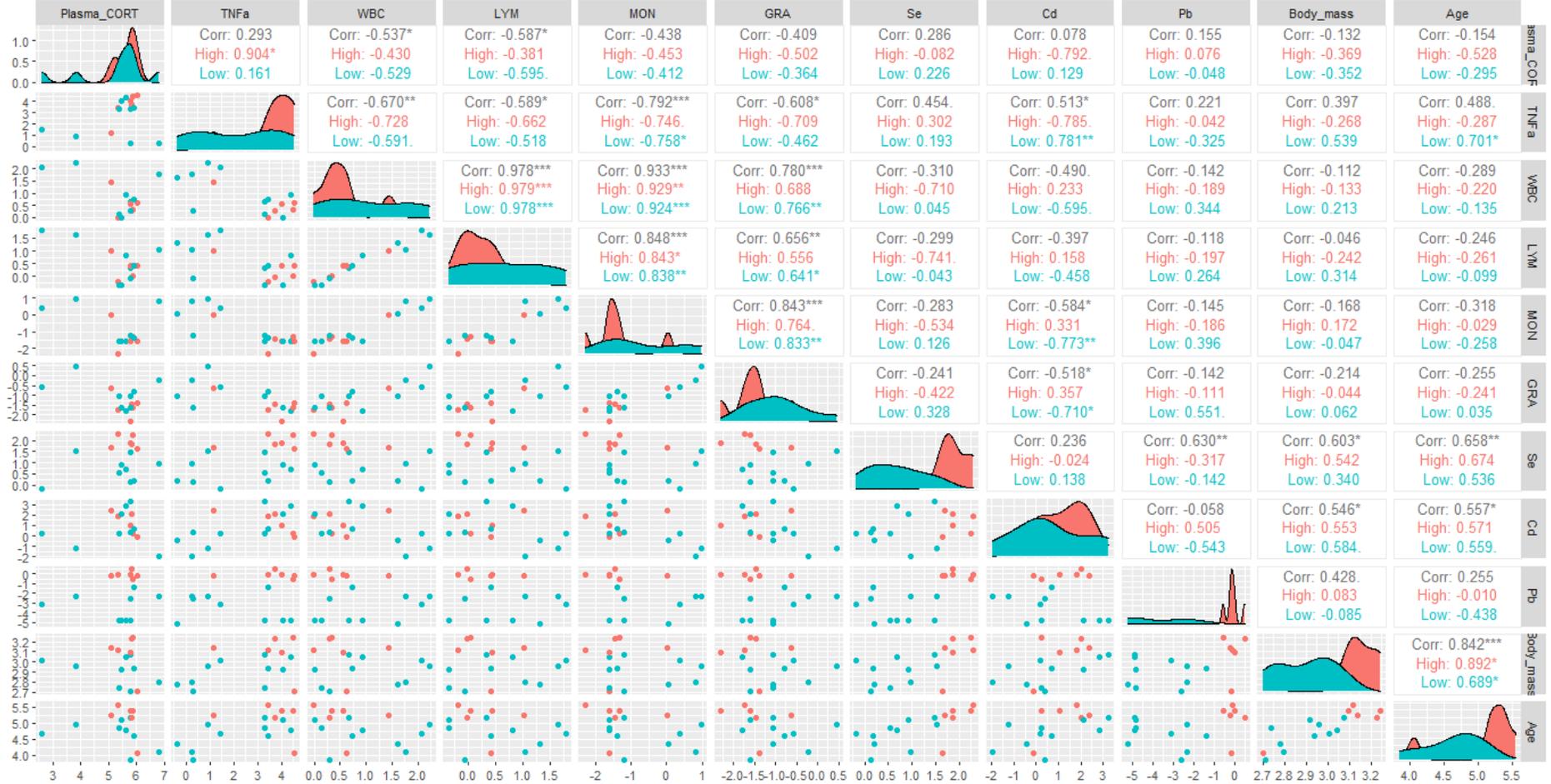
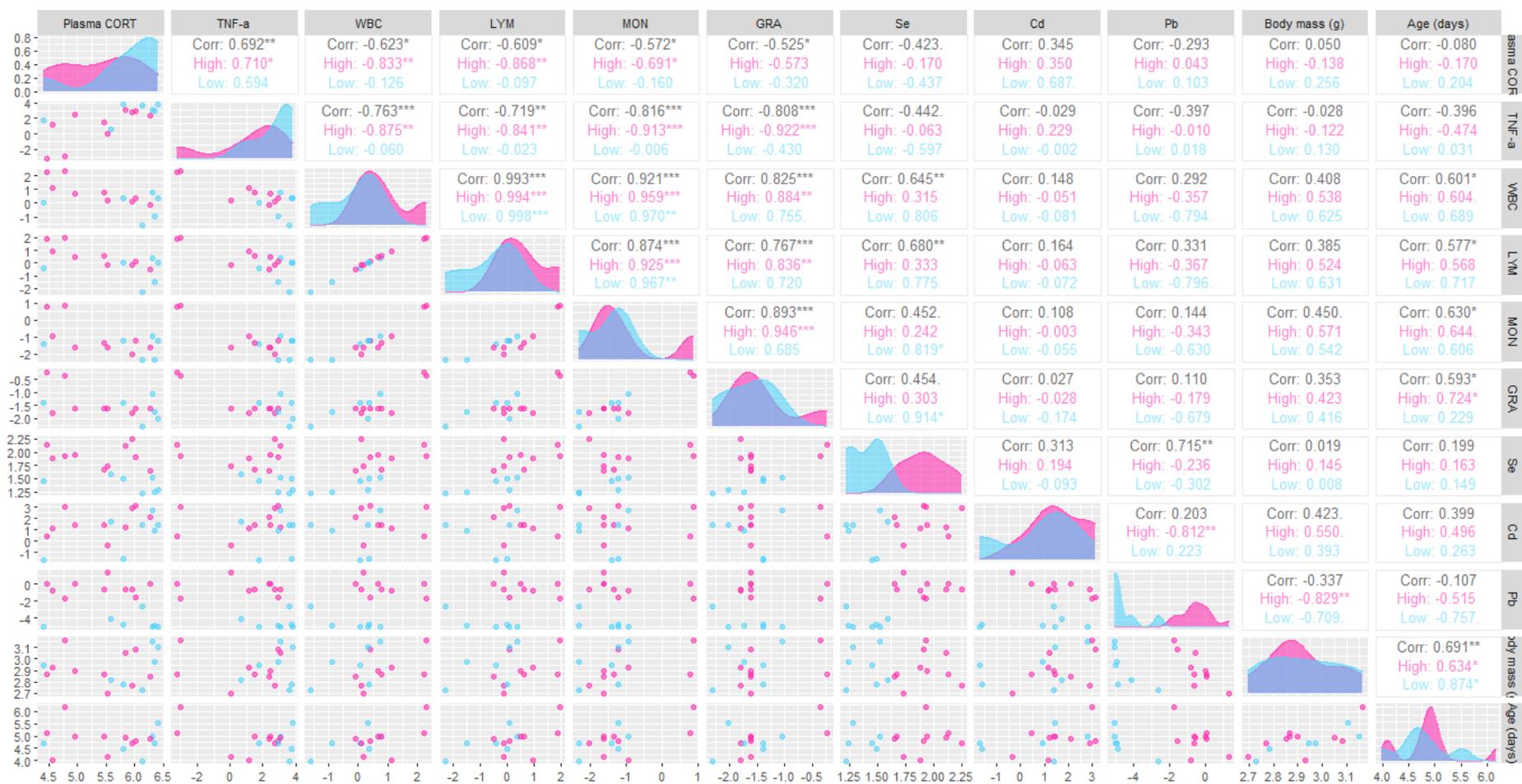
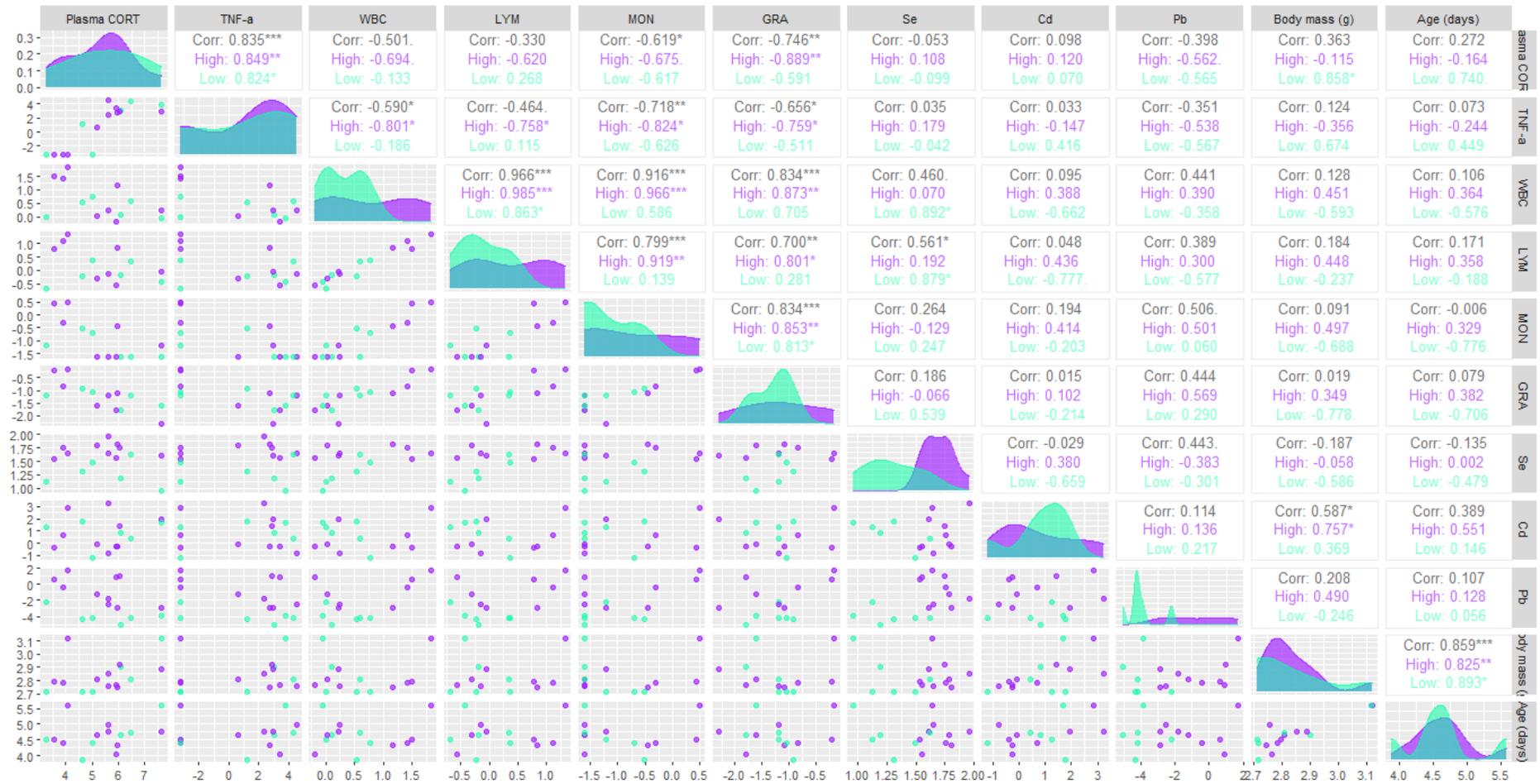


Figure S8 Correlation matrix of Spearman of the physiological variables measured 2 hours after LPS challenge (i.p. injection) in wood mice captured on contrasted levels of pollution (high and low) at 5 days of captivity with standard (A) and selenium deficient laboratory rodent diet.

A/Standard diet

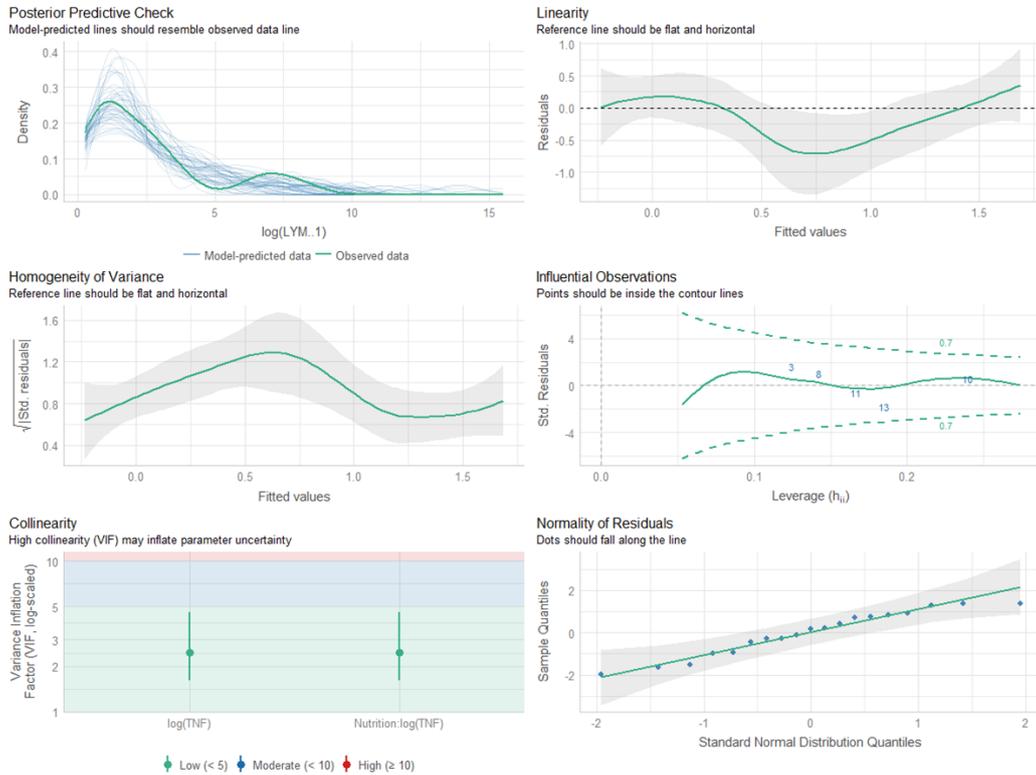


B/Deficient diet



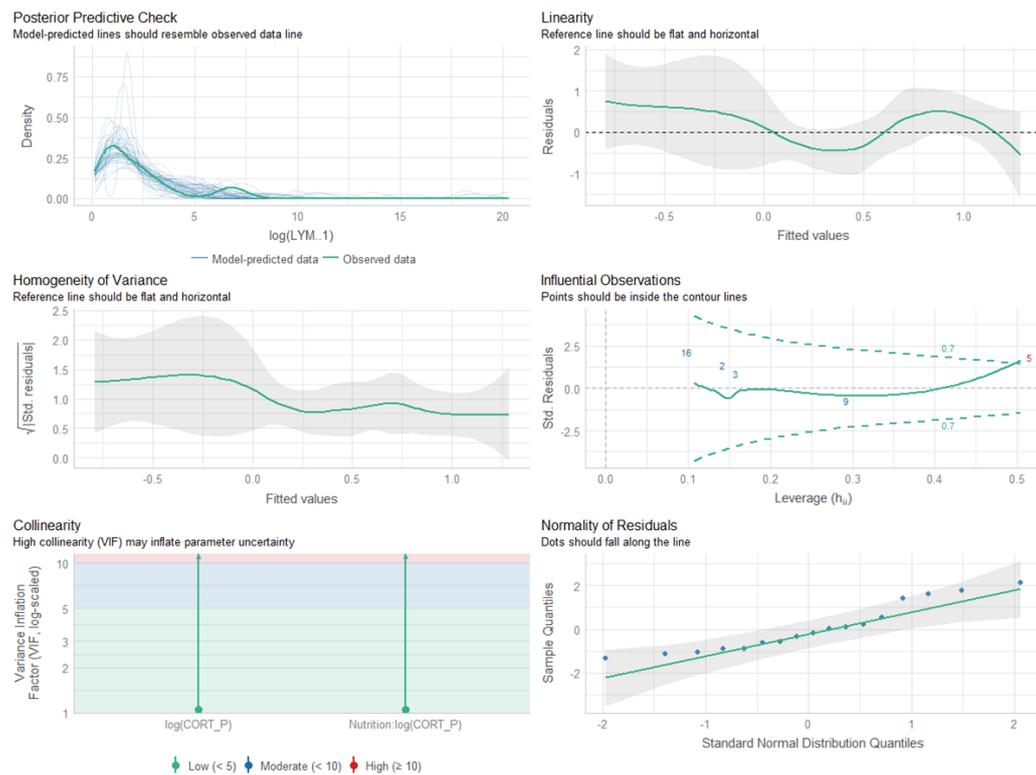
1 **Figure S9** Parameters of the linear regression models on the effect of LPS challenge in
 2 individuals from experiment 2 on LYM in function of plasma TNF- α (A) and CORT (B)
 3 concentrations in interaction with diet (models are presented in Figure 4).

A



4

B



5